Synthesis of Peptide-Derived Tetraoxazole Containing Macrocycles

by

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Abstract

Natural macrocyclic peptides isolated from marine metabolites have shown interesting biological activities, including anticancer properties. In addition, they are highly pre-organized for metal inclusion due to their structural features, such as the presence of a cavity with perfectly located oxygen and nitrogen donors within the macrocyclic structure. This thesis pertains to the development of a synthetic approach towards the synthesis of natural macrocyclic structures. These macrocyclic peptides are made up of four oxazole rings connected by amide bonds with functionalized side chains. A synthetic strategy for the synthesis of natural peptide-derived macrocyclic analogues was developed using solution phase peptide synthesis. The synthesis of the tetraoxazole macrocycle is described in more detail where the synthesis started with the formation of a dipeptide by coupling two amino acids, followed by oxazole ring formation, and subsequent formation of the di-, tri-, and tetraoxazole peptide. All of the peptide bonds were obtained using *O*-(benzotriazol-1-yl)-*N,N,N′,N′*-tetramethyluronium hexafluorophosphate (HBTU) or 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC.HCl) with hydroxybenzotriazole (HOBt) with the base N,N-diisopropylethylamine (DIEA). Finally, macrocyclization of the linear unprotected tetraoxazole gave the macrocyclic structure. The macrocyclization was achieved efficiently by using (ethyl cyano(hydroxyimino)acetato)-tri-(1-pyrrolidinyl)-phosphonium hexafluorophosphate (PyOxim) as a coupling reagent under very dilute conditions. All structures were analyzed by ^{1}H and ^{13}C NMR. With the structural similarities to the macrocyclic peptides isolated from marine sources, this tetraoxazole macrocyclic peptide might be used as a chelating agent.

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List of Abbreviations

Boc	*tert*-Butyloxycarbonyl
COSY	Correlation spectroscopy
Cys	Cysteine
DAST	Diethylaminosulfur trifluoride
DEPT	Distortionless enhancement by polarization transfer
DBU	1,8-diazabicyclo[5.4.0]undec-7-ene
DCM	Dichloromethane
DIEA	N,N-Diisopropylethylamine
DMA	N,N-Dimethylaniline
DMF	N, N-dimethylformamide
DMSO	Dimethylsulfoxide
EDC.HCl	1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride
EtOAc	Ethyl acetate
Fmoc	9-Fluorenylmethoxycarbonyl
HOBt	Hydroxybenzotriazole
h	Hour
i-PrOH	Isopropyl alcohol
MeOH	Methanol
mp	Melting point
NBS	N-bromosuccinimide
NMR	Nuclear magnetic resonance

PG	Protecting group
PyOxim	(Ethyl cyano(hydroxyimino)acetato)-tri-(1-pyrrolidinyl)-phosphonium hexafluorophosphate
rt	room temperature
Ser	Serine
SPPS	Solid-Phase Peptide Synthesis
TBS	*tert*-butyldimethylsilyl
t-Bu	*tert*-Butyl

Chapter 1- Introduction

1.1 Overview

Macrocyclic peptides, in a variety of sizes and shapes, have been isolated as secondary metabolites from natural sources such as bacteria, fungi, plants, and marine sources, and many have been proven to possess interesting biological properties. During studies conducted in the 1970s, several secondary metabolites were isolated from marine organisms with differing properties, including antitumor, antibiotic, and cytotoxic activities.[1,2]

The major drawback for studying the chemistry of the natural metabolites is the low availability of these compounds as only a small amount is available through expensive extraction processes from their natural origins. Because of the unique properties and the low availability of these metabolites, synthetic routes to the natural products and their model systems were researched to study and evaluate their pharmacological properties.[3]

1.2 Biological active macrocyclic peptides

1.2.1 Cyclosporine A

Macrocyclic peptides have been used in medicine for decades and have made significant improvements to the treatment of several diseases. Cyclosporine A is one of the natural cyclic peptides that have pharmacological properties. It is used as an immunosuppressant drug in organ transplantation in order to prevent the risk of organ rejection. It is isolated from the metabolites produced by *Tolypocladium inflatum*, and has seven *N*-methyl amino acids (**Figure 1**).[4,5]

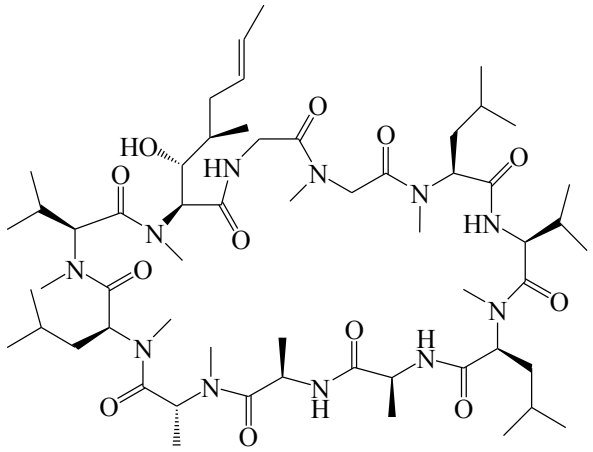


Figure 1: Structure of cyclosporine A.[4]

It was reported that cyclosporine A has one exocyclic and three endocyclic hydrogen bonds that limit the conformational freedom. It was also found that in $CDCl_3$ the hydrophobic peptide forms a twisted β-sheet with one of the *N*-methylated amide bonds. Nevertheless, it can adopt six different conformations in polar solvents such as methanol or water.[6]

In addition, when the size of a cyclic peptide increases, the conformational flexibility also rises resulting in a variety of rapidly interconverting structures. In order to restrict the conformation of the cyclic peptide, nature incorporates uncommon amino acids and constraints into the cyclic peptide in order to reduce the conformational degrees of freedom. In fact, when an amino acid adjusts to another, they can find ways to combine together to form a heterocyclic ring that is often a very active constraint. For example, the cyclooctapeptide -[L-Thr-D-Val-L-Cys-L-lle]-$_2$ depicted in **Figure 2** is flexible and can adopt a variety of conformations.[1]

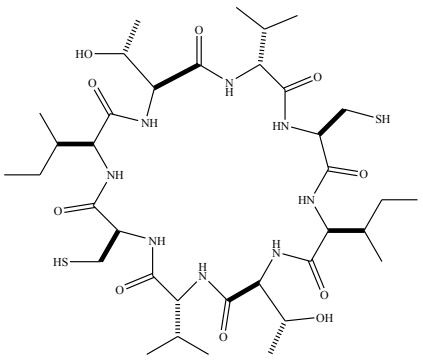


Figure 2: Structure of the cyclooctapeptide -[L-Thr-D-Val-L-Cys-L-lle]-$_2$.[1]

However, after intramolecular condensation of the threonine and cysteine side chains with the neighboring carbonyl groups, in the peptide sequence, it forms oxazoline and thiazole rings respectively, as shown for ascidiacyclamide in **Figure 3**. In this case, it only produces a single saddle shaped conformation. This conformation was confirmed via NMR spectroscopy as well as X-ray crystallography.[6]

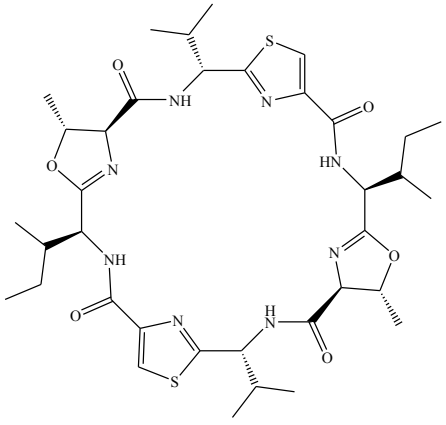


Figure 3: Structure of ascidiacyclamide.[1]

There are various biologically active macrocyclic peptides that have heterocyclic moieties in their core structure such as oxazolines, oxazoles, thiazolines, and thiazoles isolated from natural sources. These five membered heterocyclic rings are formed from the condensation of serine, threonine, and cysteine side chains with the preceding carbonyl groups in the peptide sequence.[7]

Indeed, all these moieties introduce conformational restrictions in the macrocyclic peptides making them a unique class of molecules. Some of the cyclic peptides that contain these conformational constraints have biological properties which include enzyme inhibition, antibiotic activity, antitumor activity, or anti-inflammatory activity.[6] However, the biological activity of these macrocycles in the marine organism is still unresolved. Due to the size and the conformation of these macrocycles, as well as the nature of functional groups, it might allow these macrocyclic peptides to function as metal complexation and transport agents in vivo.[8]

1.2.2 Ascidiacyclamide

Ascidiacyclamide was isolated from *Lissoclinum patella*, and it is a 24-membered azacrown-8 macrocycle (**Figure 3)**. It consists of two thiazole and two oxazoline rings connected by amide bonds with unique biological properties, such as antitumor activity. In addition, the macrocyclic peptide can be called a pseudo-octapeptide since its heterocyclic rings are derived from the condensation of two amino acid residues.[8]

It has been observed that organisms like *ascidians,* known as sea squirts, can concentrate metals, including copper, up to a concentration ten thousand times higher than that found in the local marine environment. In fact, Cu(II) ions can be observed in non-polar fractions of the sea squirt, which suggests that Cu(II) ions are complexed by organic molecules, such as the cyclic peptide ascidiacyclamide. The macrocyclic peptides are

most likely produced to coordinate with Cu(II) ions, and these Cu(II) complexes might be involved in the *ascidian's* metabolism.[8]

The saddle shaped conformation of ascidiacyclamide can contain two copper ions. These two copper ions are able to capture CO_2 to form a carbonate-bridged dinuclear Cu(II) complex. The Cu(II) ions are coordinated to three nitrogen donors; two from the heterocyclic ring and a third originating from a deprotonated amide. The coordination sphere of Cu(II) is completed by one molecule of water each as shown in **Figure 4**.[6,9]

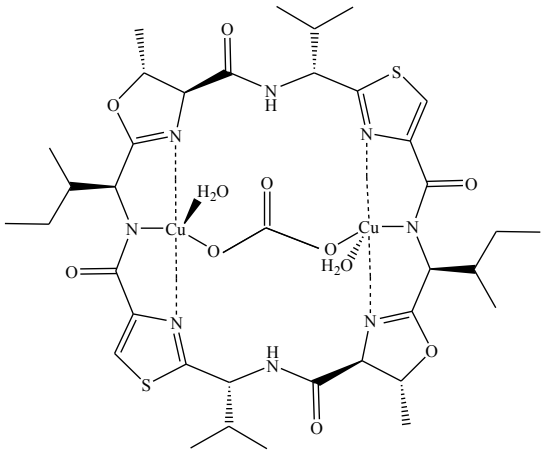


Figure 4: Structure of ascidiacyclamide complex with copper(II).[9]

The heterocyclic rings afford two of the three nitrogen donors, thus, they affect the coordination chemistry of the macrocyclic peptides and the complex stabilities. This metal complex of a marine cyclic peptide might be involved in hydrolysis and transport of CO_2 in the *ascidian's* metabolism. Other possible applications of this complexation are Cu(II) detoxification, storage or transport.[8]

1.2.3 Structural features of cyclic pseudo-octapeptides

The structural features of cyclic pseudo-octapeptides were investigated in order to explain the coordination chemistry of these cyclic peptides. Indeed, the conformations of the marine macrocyclic peptides that incorporate heterocyclic rings were studied in both solution as well as solid phases. Analysis of the solid phase of these macrocycles was carried out using X-ray crystallography while ^{1}H and ^{13}C NMR spectroscopy were used for analysis of the structures in solution. These studies afforded valuable information that explains how the complexation might influence the reactivity and the biology of these metabolites.[8]

There are many factors that can affect the structure of cyclic pseudo-peptides, such as the amino acid residues that connect the heterocyclic rings, the configuration of amino acids (R or S), and the nature of incorporated heterocyclic rings. The macrocyclic peptides adopt inflexible structures when the macrocyclic ring closes. Furthermore, incorporation of unsaturated heterocycles lowers the flexibility of the macrocyclic peptides and the planarity of the heterocycle is increased to the adjacent peptide group.[8]

The cyclic pseudo-octapeptides can adopt a variety of conformations depending on the connecting amino acids.[10] As aforementioned, symmetrical peptides like ascidiacyclamide take on a saddle-shaped conformation also referred to as a square conformation, where the oxazoline and thiazole rings are placed alternately at each corner of the rectangular ring, and the eight nitrogen atoms point toward the inside of the molecular structure. Conversely, asymmetrical peptides such as patellamide D (**Figure 5**), which is another natural cyclic peptide isolated from *Lissoclinum patella*, adopt a figure eight conformation as shown in the **Figure 6**.

This conformation is stabilized by intramolecular hydrogen bonds as well as by π-stacking of its heterocyclic rings. This conformation is stabilized by two trans-annular N-H….O=C (amide) and two trans-annular N-H….O_{oxa} (oxazoline ring) hydrogen bonds; see **Figure 7** for nomenclature. Moreover, the nitrogen atom in the oxazoline rings point toward the outside of the macrocyclic peptide in this conformation.[11,12]

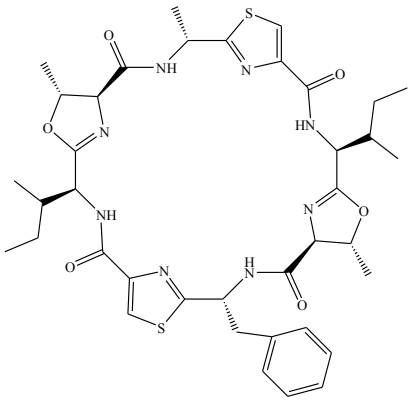
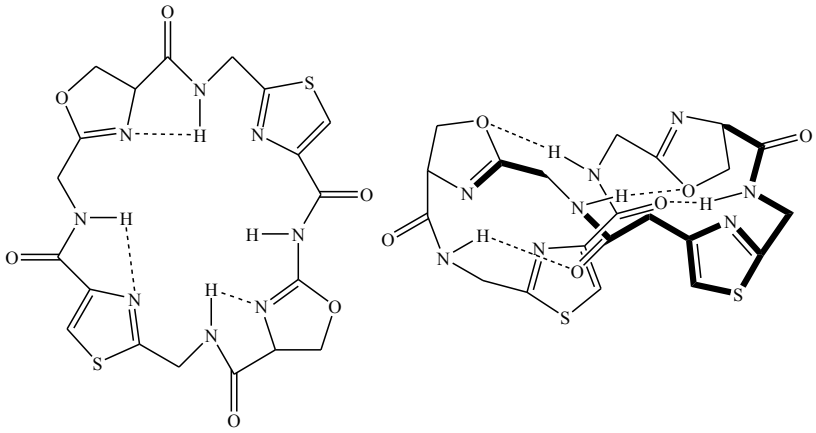


Figure 5: Structure of patellamide D.[12]



square/saddle shaped conformation

figure eight conformation

Figure 6: Conformations of cyclic pseudo-octapeptides (ascidiacyclamide and patellamide): square and figure eight.[12]

Usually, all of the protons from the amide bonds point towards the center of the macrocycle whereas the carbonyl groups point towards the outside of the macrocyclic peptide. In addition, the amino acid side chains adopt axial positions. In the case where all the amino acid residues that connect the heterocycles have identical configuration (*L*- or *D*-), all the amino acid residues face the same side of the macrocycle. On the other hand, if the cyclic pseudo-peptides are built up from *D*- and *L*-amino acids, the macrocyclic peptides can exhibit an alternating stereochemistry in the linkers like ascidiacyclamide. Furthermore, the incorporation of oxazoline rings produces an almost coplanar structure of the heterocyclic moieties of the macrocyclic peptides whereas those peptides that contain oxazole would have a cone-like structure.[11]

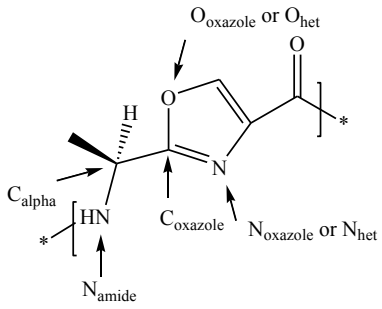


Figure 7: Nomenclature of the heterocyclic blocks of the macrocycle.[11]

1.2.4 Westiellamide

Westiellamide is a cyclic pseudo-hexapeptide isolated from the ascidian *Lissoclinum bistratum*. As in the bigger 24-membered azacrown-8 macrocycles, westiellamide, an 18-membered azacrown-6 macrocycle, is highly preorganized for metal complexation. This compound has three oxazoline rings that are connected by amide bonds in a preorganized arrangement (**Figure 8**).[13,14]

Based on the study by Comba and co-workers[15], westiellamide forms stable mononuclear and dinuclear Cu(II) complexes. The formation of complexes involves metal-ion-assisted deprotonation of amide bonds, providing nitrogen donors. Moreover, in mononuclear complexes, Cu(II) ion prefers to bind to a $N_{heterocycle}$-N_{amide}-$N_{heterocycle}$ binding site. However, in the methoxide or hydroxide-bridged dinuclear complexes, the second Cu(II) ion is coordinated to a N_{amide}-$N_{heterocycle}$-N_{amide} binding site.

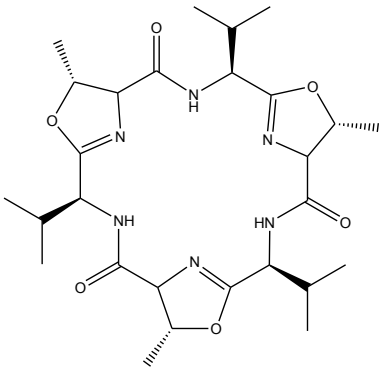


Figure 8: Structure of Westiellamide.[13]

Furthermore, systematic metal-ion-binding studies with westiellamide have reported the affinity of Ag(I). Initial studies on the metal complexation of westiellamide were conducted by Wipf and co-workers who described a unique Ag_4 cluster complex with westiellamide (**Figure 9**).[16] In fact, three Ag(I) ions are organized in a pseudo trigonal arrangement around the central silver ion Ag(I) and are bordered on two sides by two macrocycles. The central silver ion Ag(I) is coordinated in an octahedral arrangement by the carbonyl oxygen atoms of the two westiellamide macrocycles.

1.2.5 Structural feature of cyclic pseudo-hexapeptide

The configuration of westiellamide is S where the *L*-valine's side chains are all on one side of the macrocyclic peptide, and the amide and heterocyclic nitrogen donor atoms point toward the inside of the macrocycle.[13] Moreover, the metal free macrocycle westiellamide, which is a symmetrical cyclic peptide, adopts a triangular configuration. Indeed, the heterocyclic nitrogen atoms as well as the amide hydrogen atoms point towards the inside of the macrocycle. Nevertheless, this conformation is twisted in the silver cluster. The carbonyl groups of westiellamide are rotated towards the inside of the macrocycle in order to coordinate with the central Ag(I) ion, and at the same time rotate the amide nitrogen atoms towards the outside of the westiellamide. The x-ray structure shows an unusual feature of the sandwich complex where the central Ag(I) is coordinated octahedrally by carbonyl oxygen atoms of the two-westiellamide macrocycles.[17]

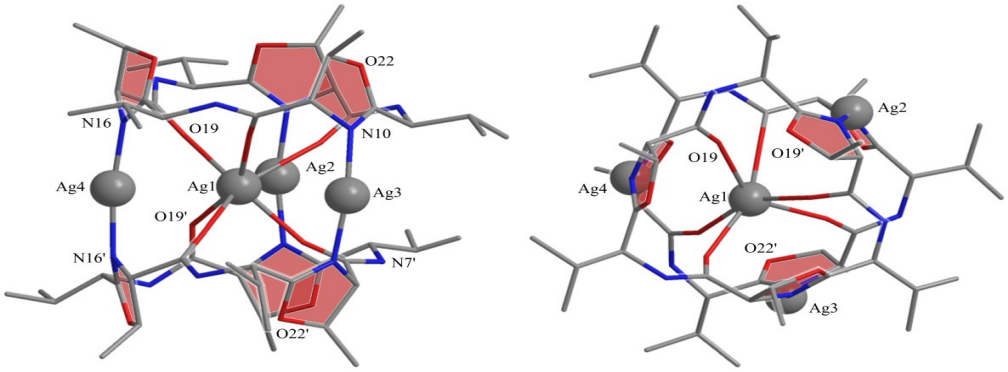


Figure 9: X-ray structure of the silver(I) complex of westiellamide.[8]

1.2.6 Telomestatin

Telomestatin is a natural product that has been isolated from *Streptomyces anulatus* 3533-SV4, whose structure is made up of a macrocycle containing linkage of two methyl

oxazoles, five oxazole rings, and one chiral thiazoline ring as shown in the **Figure 10**. Telomestatin is one of the most potent telomerase inhibitors reported to date. Due to the high affinity of telomestatin for intramolecular G-quadruplex structures, it can inhibit the cell growth in cancer cells.[18] Telomestatin is involved in the selective binding of G-quadruplex structures, and the high affinity and specificity of telomestatin for G-quadruplex is justified by its size as well as shape complementarity with G-tetrads of G-quadruplex DNA.[19]

It was also found that telomestatin has the affinity to coordinate with cations such as K^+ and Na^+, and this feature could affect its binding to G-quadruplex DNA.[20]

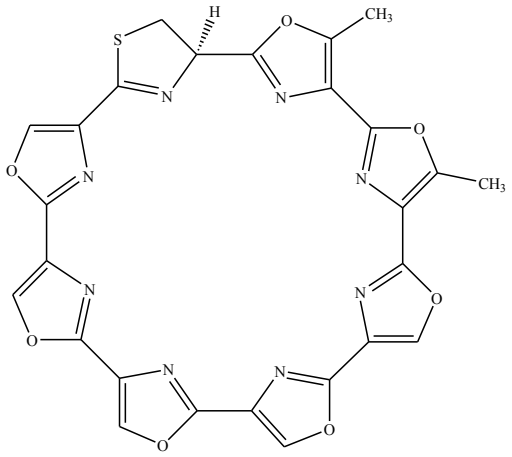


Figure 10: Structure of Telomestatin.[18]

One main drawback of telomestatin is the difficulty in obtaining it from natural sources. The amount that has been isolated from the bacteria is very low. In addition, the total synthesis of telomestatin was described in a study conducted by Takahashi et al., (2006) however, their method is not sufficient for large-scale synthesis, and is not suitable for the synthesis of derivatives. [21]

1.3 Metal ion coordination to cyclic peptides in nature

Many of the marine macrocycles such as ascidiacyclamide, and westiellamide consist of a macrocyclic cavity with polar functional groups. This together with the capacity of *ascidians* to concentrate metal ions suggests that the macrocycles are made to bind with metal ions. The geometric arrangement of the functional groups in the macrocyclic peptides, the set of nitrogen donors in the macrocycles, as well as the size of these compounds suggests that their biological functions are to coordinate with metal ions. The purpose of this macrocyclic complexation might be responsible for either metal transport, biological assembly of the metabolites, or catalysis.[8,15]

1.4 Advantages of macrocyclic peptides over linear peptides

Peptides show several advantages as components of drugs, such as low toxicity, since they are made up of amino acids. However, linear peptides adopt a variety of conformations in solution. This conformational flexibility of linear peptides often leads to poor selectively against biological targets.[22] In addition, linear peptides are highly degraded by peptidases, which leads to low bioavailability. One method to reduce the flexibility of peptides, and instability to peptidases, is the cyclization of the linear peptides. Cyclization of a linear peptide is a useful strategy to limit the conformational freedom thereby increasing the binding affinity as well as the selectivity for the biological target.[23]

The hydrophobic side chains of macrocyclic peptides afford a hydrophobic surface that shields or protects their cleavable amide bonds from degradation by peptidases. This hydrophobic surface might also facilitate their penetration into cell membranes. Therefore, increasing resistance to degradation by peptidases leads to longer lifetimes in biological systems. Furthermore, because the macrocyclic peptides impose limitations to conformational flexibility, they are capable of better selective interactions with bioreceptors, compared to the flexible linear peptides. Thus, the macrocyclic peptides are usually less toxic than the linear peptides.[6]

1.5 Synthetic routes to peptide-derived macrocycles

In general, the synthesis that is used to create these types of peptide-derived macrocycles is made up of three parts. First, the heterocyclic building block, made up of an amino acid linker and a heterocyclic ring, is synthesized. Second, convergent or stepwise coupling of the building blocks is achieved in order to obtain the desired linear peptide, with heterocyclic rings. The last step is the macrocyclization under dilute conditions to avoid intermolecular coupling and induce the preferred intramacrocyclization.[24,25,3]

Symmetrical macrocyclic structures can be created by forming dimers and tetramers, from the oxazole building block, followed by macrocyclization. Asymmetrical macrocyclic structures might need the synthesis of a different linear sequence, with a subsequent macrocyclization.[26]

1.6 Synthesis of macrocyclic peptides

Depending on the amino acid's functional group, there are four different strategies that can be used to transform a peptide into a macrocycle. The first of these strategies is head-to-tail cyclization where the amide bond forms between the carboxyl and the amino groups at the *C*- and *N*-terminus. The second strategy is a side chain-to-tail cyclization where the peptide bond forms between the amino group at the *N*-terminus and the carboxyl group of the side chain. The third strategy is a head-to-side chain cyclization, where peptide bond formation is achieved with the amino group of the side chain and the carboxylic group of the *C*-terminus. Finally, there is the side-chain-to-side-chain cyclization where a disulfide bond or bridge forms from the two cysteine residues as seen in **Figure 11**.[27] Scientists have used each of these methods to form macrocyclic peptides, but the two most commonly employed methods are head-to-tail and side-chain-to-side-chain cyclization.[27] The former is the most common strategy used for cyclizing peptides in solution phase peptide synthesis. In addition, cyclization in solution phase is faster and provides the final cyclic peptide in a high yield.[22]

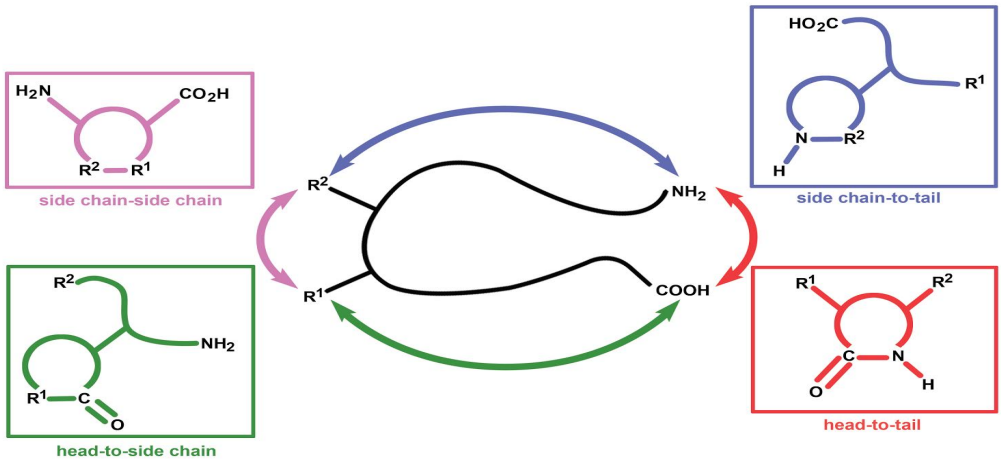


Figure 11: Four different ways to constrain a peptide into a macrocycle (figure adapted from reference 26).

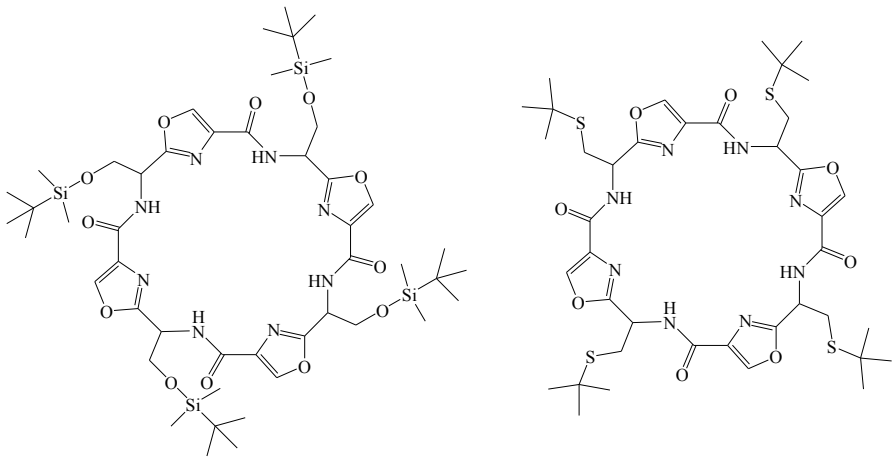
It is preferred that macrocyclizations be done under high dilution in order to minimize the intermolecular side reactions, giving rise to oligomers and polymers of the peptide. Furthermore, the cyclization of a peptide depends on the ability of the linear peptide to prearrange its two reactive terminals within close proximity of one another. The prearrangement leads to efficient macrocyclization with fewer side-reactions resulting from intermolecular reactions.[27]

1.7 Objective

Macrocyclic peptides that have been isolated from marine sources have shown interesting metal complexation patterns. The structures of these macrocycles are highly preorganized for optimal metal ion complexation, in terms of their ring structure, the size of the macrocyclic peptide, and the set of the amide bonds as well as the nitrogen donor atoms in the heterocyclic rings.

This thesis deals with the synthesis of new tetraoxazole macrocyclic peptides (**Figure 12**) which mimic the structure of the natural macrocycles. The macrocyclic peptides designed are analogues of the marine natural products, which contain four oxazole rings connected by amide bonds and functionalized with hydrophobic amino acid residues, such as *tert*-butyldimethylsilyl (TBS) or *tert*-butyl (*t*-Bu) groups. This thesis presents the design as well as the methodology to create these macrocyclic peptides through solution phase peptide synthesis.

In addition, towards the synthesis of these macrocyclic peptides, new synthetic methodologies will be developed including the use of silyl-protected alcohols for cyclodehydration reactions, as well as the synthesis of linear di-, tri-, and tetraoxazole peptides.



Tetra-oxazole macrocyclic peptide with TBS groups Tetra-oxazole macrocyclic peptide with *t*-Bu groups

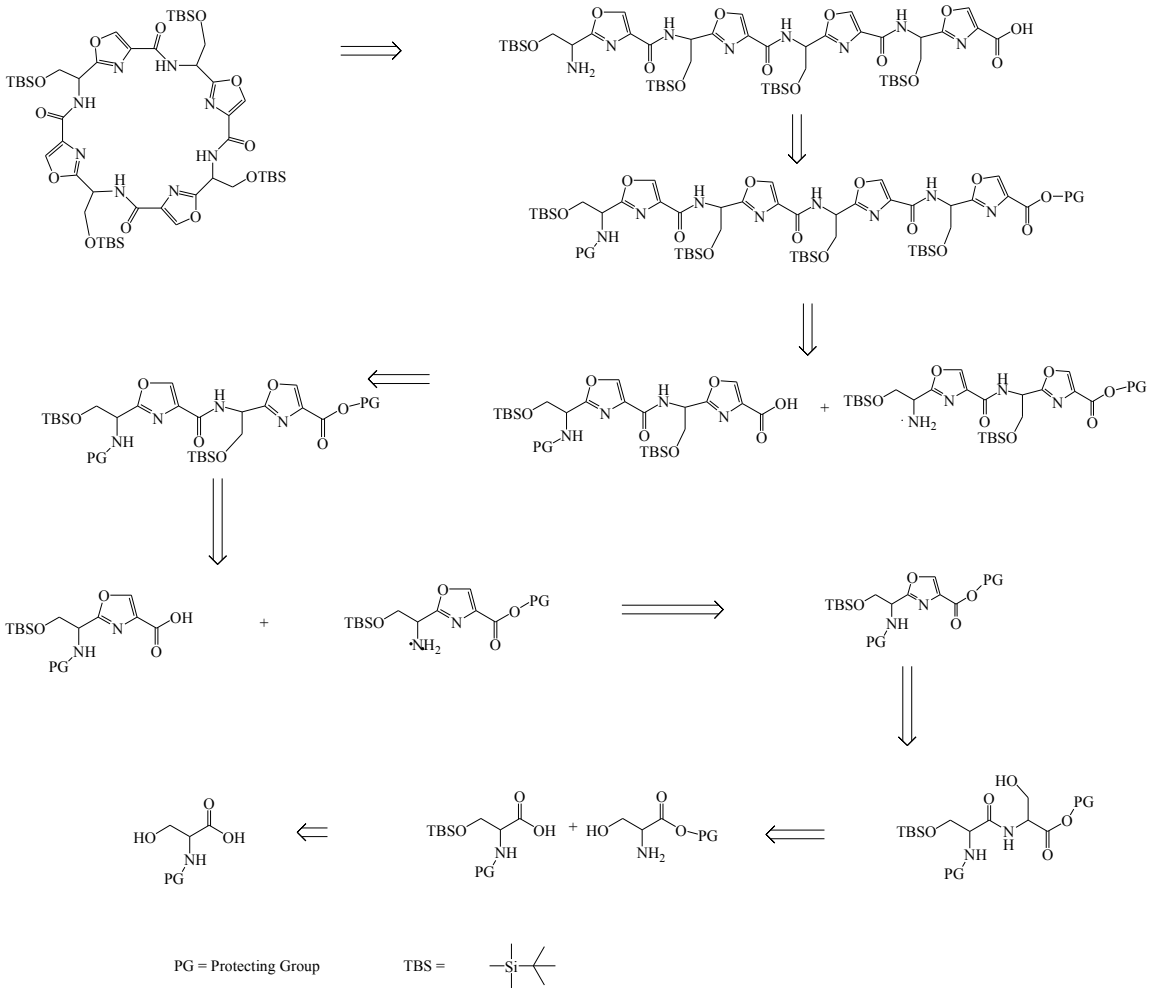
Figure 12: The tetraoxazole macrocyclic peptides.

Chapter 2- Result and discussion

2.1 Retrosynthetic approach towards amino acid-derived tetraoxazole macrocyclic structures

The retrosynthetic analysis of the target molecule (tetraoxazole macrocyclic peptide), shown in **Scheme 1,** involves a convergent synthesis using a solution phase peptide approach. Because the desired compound is symmetric, tetraoxazole can be made using a short synthetic route. Once the monooxazole is obtained, from the cyclodehydration and oxidation of a diserine, the dioxazole can be produced by dividing the amount of the monooxazole in half. One half of the monooxazole can be used to deprotect the *N*-terminus and the other portion to deprotect the *C*-terminus, thus allowing for the selective coupling of these peptides to get the dioxazole. The three steps can be repeated in order to obtain the tetraoxazole.

The desired octapeptide (tetraoxazole) macrocycle was synthesized by a head-to-tail peptide coupling of the linear tetraoxazole precursor at very dilute concentrations. The concentration is critical in the synthesis of macrocyclic peptide to minimize the formation of oligomers. The linear tetraoxazole was generated by full deprotection of the protected tetraoxazole peptide. The tetraoxazole was obtained by coupling two dioxazoles, and the dioxazole precursor was synthesized by coupling two monooxazoles. In addition, monooxazole was generated by cyclodehydration followed by oxidation of the dipeptide, which was generated by coupling two protected L-serines where PG is the abbreviation used for used for protecting group. Thus, three protecting groups were used in the synthesis of tetraoxazole macrocyclic peptide: fluorenylmethyloxycarbonyl (Fmoc) group to protect the amine; methyl ester (OMe) group to protect the acid; and TBS group to protect the hydroxyl group of Ser.



Scheme 1: Retrosynthesis of the tetraoxazole macrocyclic peptide containing the TBS protected L-serine residue.

It is known that the Fmoc group can be removed by using $AlCl_3$ and N,N-dimethylaniline (DMA) to get the free amine.[28] Methyl ester hydrolysis can be achieved by using NaOH and $CaCl_2$ in *i*-PrOH/H_2O to give the free acid.[29]

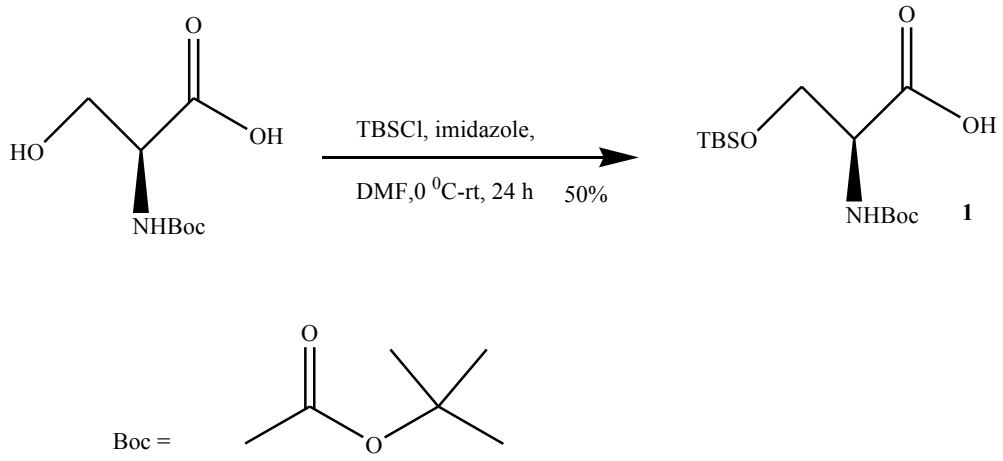
It was preferred to use solution phase peptide synthesis because the intermediates can be isolated and characterized after each step. It is easy to detect the products of side reactions and remove unwanted by-products prior to the next reaction.[30] In contrast, solid phase peptide synthesis (SPPS) does not allow for immediate analysis of the intermediate

peptides because the peptide is attached to an insoluble polymer. Solution phase peptide synthesis is mostly used for large-scale peptide synthesis whereas SPPS is limited to the milligram scale. In addition, the synthesis of peptides made up of unusual amino acids such as oxazoline and oxazole, and the synthesis of cyclic peptides can be achieved through solution synthesis.[30,31]

2.2 Preliminary synthetic studies towards the tetraoxazole macrocyclic peptide using a Boc protecting group

2.2.1 Protecting the side-chain of L-serine with TBS (synthesis of Boc-Ser-OH(O-TBS))

The synthesis of the tetraoxazole macrocyclic peptide requires the coupling reaction of several amino acid subunits, and in order to avoid side reactions, the amino acid side-chain should be protected. The hydroxyl group of the side chain of L-serine can be protected as a silyl ether. The TBS group was chosen to protect the hydroxyl group because it is stable in alkaline solutions which make it ideal for subsequent reactions that need basic conditions. In addition, to the best of my knowledge, there are no reports on macrocyclic peptides where the side chains are protected with TBS groups. Hence, it was of interest to study the characteristics of this macrocycle such as complexation with metal ions. The protection can be accomplished by using TBSCl as a reagent with imidazole as a catalyst in DMF (**Scheme 1**).[32,33] The synthesis of Boc-Ser-OH(O-TBS) **(1)** was achieved by adding TBSCl and imidazole to a solution of Boc-L-Ser in DMF at 0 ^{0}C-rt for 24 h. The Boc-Ser-OH(O-TBS) was obtained as a colorless oil in 50% yield. The structure was confirmed by ^{1}H NMR and ^{13}C NMR (see Appendices 1 and 2).

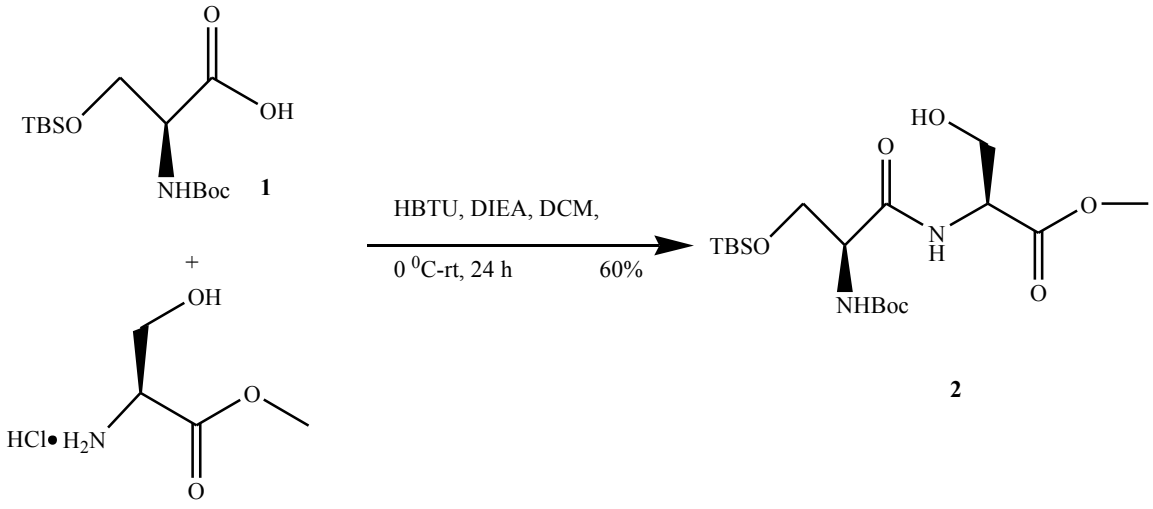


Scheme 2: Synthesis of Boc-Ser-OH(O-TBS).

2.2.2 Synthesis of the protected dipeptide Boc-Ser(O-TBS)-Ser-OMe

The protected dipeptide Boc-Ser(O-TBS)-Ser-OMe was obtained by coupling the free acid Boc-Ser-OH(O-TBS) with the amine HCl.H_2N-L-Ser-OMe. The coupling reagent *O*-(benzotriazol-1-yl)-*N,N,N′,N′*-tetramethyluronium hexafluorophosphate (HBTU) was used in order to facilitate the peptide bound formation in the coupling reaction because it is fast, complete, free of racemization, and proceeds with minimal side reactions.[34,35] This reagent reacts with the carboxylic acid to form an active ester that can react with the free amine to form the amide bond. Furthermore, HBTU reacts with amino groups, forming tetramethylguanidinium derivatives. Therefore, permanent blocking of the *N*- terminal amino group can occur when HBTU is added directly to the amino compound. This blocking can be avoided by activating the amino acid to be coupled with the coupling reagent before adding the amino acid with a free amino group.[36]

Utilizing the free acid Boc-Ser-OH(O-TBS) **(1)**, the amine as a salt HCl.H_2N-L-Ser-OMe, HBTU and N,N-diisopropylethylamine (DIEA) as a base, the dipeptide Boc-Ser(O-TBS)-Ser-OMe **(2)** was synthesized in DCM as shown in the **Scheme 3**.[37] The reaction was monitored via TLC, and upon completion, the reaction was worked up by an acid-base extraction. The crude dipeptide was crystallized with hot hexane to give the pure dipeptide Boc-Ser(O-TBS)-Ser-OMe **(2)** as a yellow solid in 60% yield. The structure of the pure dipeptide was confirmed by ^{1}H and ^{13}C NMR (see Appendices 3 and 4).

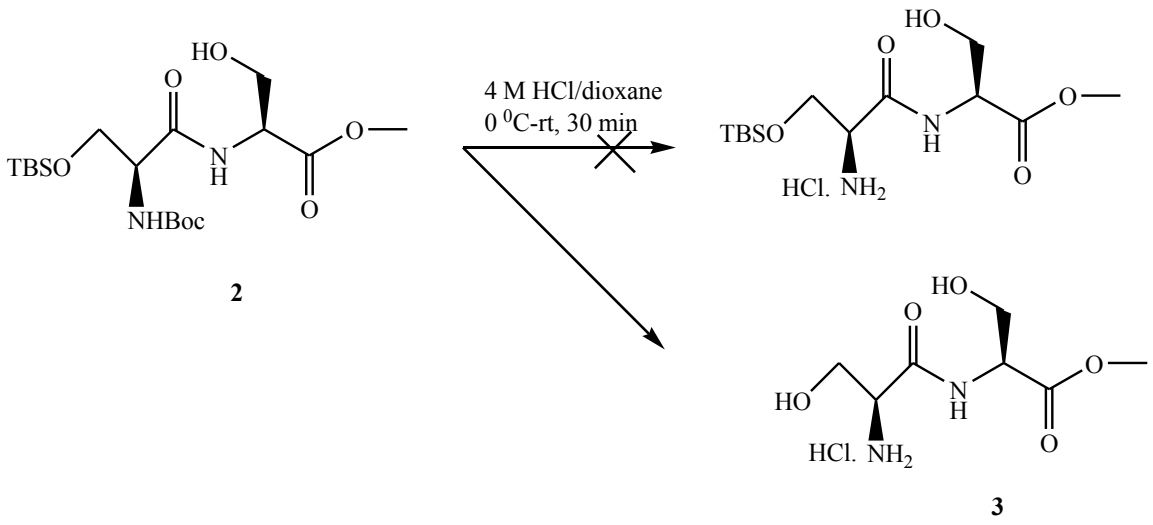


Scheme 3: Synthesis of Boc-Ser(O-TBS)-Ser-OMe.

2.2.3 Selective Cleavage of the Boc group without affecting the TBS group

Before proceeding further in the synthesis of the monooxazole, the method of using Boc and TBS groups on the dipeptide was evaluated since both of the protecting groups are acid labile. Cavelier and Enjalbal reported an interesting selectivity of Boc removal in the presence of an acid labile TBS group using saturated HCl in dioxane.[38] According to the

literature, a Boc group can be selectively removed in the presence of TBS ether using saturated HCl in either 1,4 dioxane or ethyl acetate without affecting the TBS group in the Boc-protected peptide, Boc-Leu-Tyr(TBS)-OMe. However, when the experiment was carried out using 0.2 mmol of the dipeptide **(2)** in 4 M HCl in dioxane (16 mmol),[39] both of the protecting groups, Boc and TBS, were cleaved. In fact, 4 M HCl in dioxane was cooled to 0 ^{0}C using an ice-water bath under an argon atmosphere. Following this, the dipeptide was added in one portion at 0 ^{0}C and the ice bath was removed, and the mixture was stirred for 30 min at room temperature. The reaction was monitored via TLC, and upon completion, the mixture was concentrated *in vacuo* and the residue was washed with dry ether. The ^{1}H NMR and ^{13}C NMR (see Appendices 5 and 6) analysis of the dipeptide salt indicated the removal of both protecting groups, Boc as well as TBS (**Scheme 4**).



Scheme 4: Attempted removal of the Boc group from the dipeptide Boc-Ser(O-TBS)-Ser-OMe.

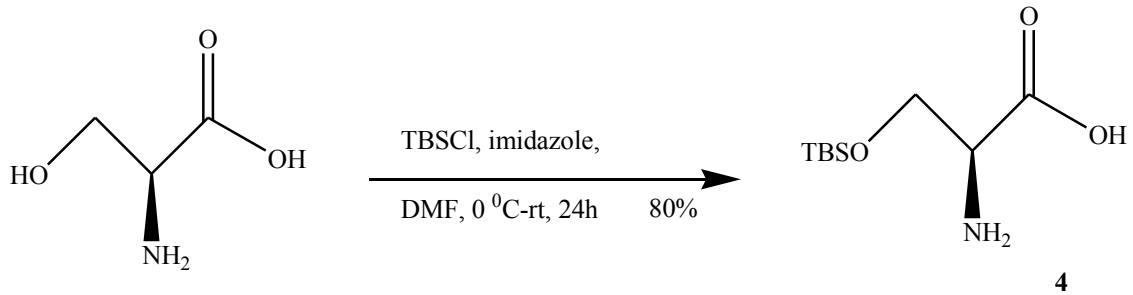
For this reason, the experimental conditions such as the reaction temperature, and the reaction time were altered to see if the result might change. The reaction was run again for about 15 min at room temperature. Unfortunately, the same result was obtained since the loss of the silyl ether group occurred before the Boc deprotection as indicated from

the NMR data. The selectivity of the Boc removal in the presence of TBS group using 4 M HCl in dioxane depends on the nature of the dipeptides, and it was not achieved in case of the primary alcohol silyl protection. Cavelier and Enjalbal reported the selective removal of Boc group was only achieved when the protected alcohol was phenolic.[38] For that reason, the protecting group (Boc) needed to be changed to another group that is not acid labile, so it can be removed selectively without affecting the TBS group. One such group could be the Fmoc group.

2.3 Synthesis of the tetraoxazole macrocyclic peptide with TBS groups using a Fmoc protecting group

2.3.1 Synthesis of Fmoc-Ser-OH(O-TBS)

Synthesis of the macrocyclic peptide began by first converting the side chain (hydroxyl group) of L-serine to a TBS ether. The TBS group was first introduced to the amino acid serine Ser-OH with TBSCl and imidazole as a base to give Ser-OH(O-TBS) **(4)** in excellent yield 80% (**Scheme 5**).[40] The structure was confirmed via ^{1}H NMR and ^{13}C NMR (see Appendices 7 and 8).



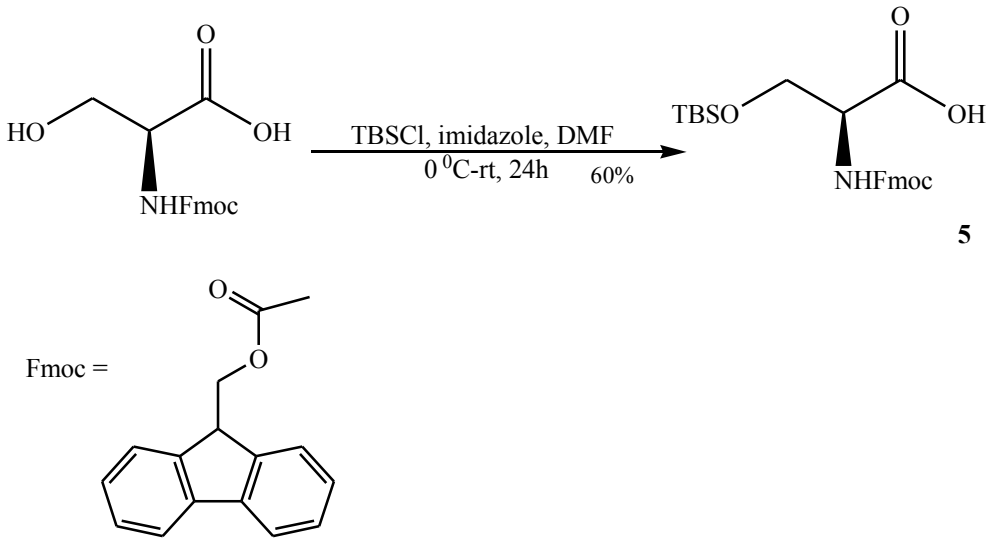
4

Scheme 5: Synthesis of Ser-OH(O-TBS).

After the protection of the hydroxyl group, the Fmoc group was used to protect the amine group in Ser-OH(O-TBS) **(4)** to afford Fmoc-Ser-OH(O-TBS). The Fmoc group was

introduced with 9-fluorenylmethoxycarbonyl chloride (Fmoc-Cl) as a reagent in the presence of Na_2CO_3,[41] but unfortunately the reaction did not yield the desired product. The Fmoc group was attached to the amine group, but the TBS group was lost. One contributing factor could be the formation of HCl, which may have the potential to cleave the TBS group.

Because of the difficulty to protect the amine with the bulky Fmoc group, the protection sequence was reversed. The TBS group was introduced to the commercially available Fmoc-L-serine. From this introduction, the reaction of Fmoc-L-Ser with TBSCl and imidazole in DMF gave the product Fmoc-Ser-OH(O-TBS) **(5)** in 60% yield along with unreacted starting material Fmoc-Ser-OH (**Scheme 6**).[42] The structure was confirmed via ^{1}H NMR and ^{13}C NMR (see Appendices 9-12).

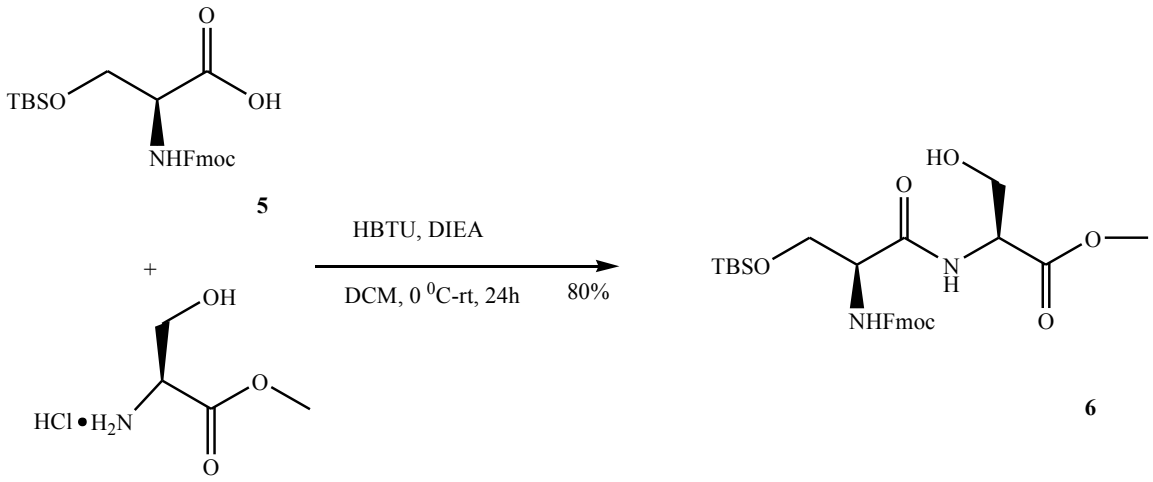


Scheme 6: Synthesis of Fmoc-Ser-OH(O-TBS) from Fmoc-Ser-OH.

2.3.2 Synthesis of Fmoc-Ser(O-TBS)-Ser-OMe

After the protection of the serine side chain, a peptide coupling reaction with serine methyl ester was used to generate the protected dipeptide. The coupling reagent HBTU

was utilized to facilitate the peptide bond formation. The dipeptide Fmoc-Ser(O-TBS)-Ser-OMe **(6)** was generated by using the free acid Fmoc-Ser(O-TBS) **(5)**, the hydrochloride of H_2N-Ser-OMe at 0.1 M concentration, the coupling reagent HBTU and DIEA in DCM (**Scheme 7**).[37] The work up was done via an acid-base extraction, followed by purification through crystallization with hot hexane to obtain the dipeptide as a white solid in 80% yield. As a result of this purification process, the tetramethylurea by-product was removed from the dipeptide. The structure of the pure dipeptide, Fmoc-Ser(O-TBS)-Ser-OMe **(6)**, was confirmed by ^{1}H NMR and ^{13}C NMR (see Appendices 13-17).



Scheme 7: Synthesis of the dipeptide Fmoc-Ser(O-TBS)-Ser-OMe.

2.3.3 Synthesis of heterocyclic oxazole ring

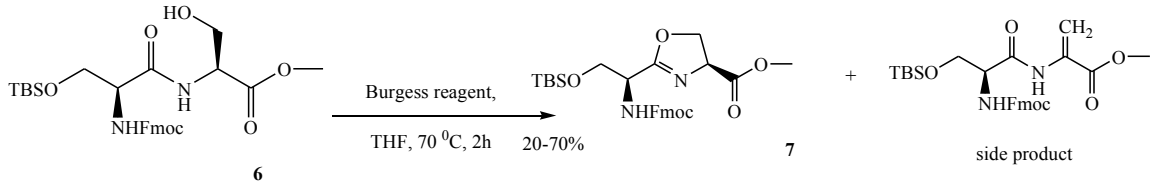
The heterocyclic ring, oxazole, is formed from the condensation of the serine side chain with the preceding carbonyl group in the peptide sequence. The most extensively used method for oxazole formation nowadays involves two steps, the first being the formation of the intermediate oxazoline. This can be generated by the activation of β-hydroxyl

amide from the dipeptide, which is required for cyclization to take place. The activation can be achieved by using dehydrating reagents such as methyl *N*-(triethylammoniumsulphonyl)carbamate (Burgess reagent)[43,44] or diethylaminosulphur trifluoride (DAST) reagent in a manner similar to that previously reported.[45,46] The second step is the oxidation of the intermediate oxazolines to provide the desired oxazoles.

2.3.3.1 Oxazoline formation

2.3.3.1.1 Reaction using Burgess reagent

The formation of the intermediate oxazoline was initially attempted by the cyclization of the β-hydroxy amide with the Burgess reagent. It is a mild and selective dehydrating reagent that was used by Peter Wipf in the formation of 5-membered heterocycles from their acyclic precursors.[43,44] In order for cyclization to occur, the activation of the hydroxyl amide is required. To explain, the synthesis of oxazoline was achieved by adding the Burgess reagent to the protected dipeptide (0.1 M) **(6)** dissolved in anhydrous THF (**Scheme 8**). The reaction mixture was refluxed at 75 ^{0}C, and TLC was used to monitor the reaction. After a 2 h time period the reaction was complete with all starting material having disappeared. The solvents were evaporated under reduced pressure to give the crude product that was subsequently purified with column chromatography. The pure Fmoc-oxazoline-OMe **(7)** was obtained as a white solid in a low yield of around 25%, and the structure was characterized by ^{1}H and ^{13}C NMR (see Appendices 18-22).



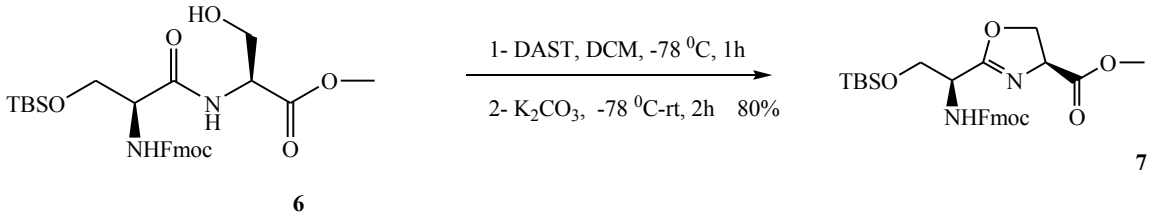
Scheme 8: Synthesis of Fmoc-oxazoline-OMe using Burgess reagent.

The low yield of the oxazoline was due to the formation of the opened chain dehydration product (**Scheme 8**) as a by-product as indicated by NMR analysis. In addition, the intermediate, oxazoline, was decomposed through the purification by column chromatography as well as in organic solution. Decomposition of the oxazoline was noticed during the purification as indicated by TLC, and was hence the reason for the low yield. It was also observed in other studies done by Miller and co-workers that oxazoline is slightly unstable and it decomposed during column chromatography.[47] Therefore, dry column chromatography was done on the crude mixture and the solvents were evaporated *in vacuo* right away to avoid being in solution to give the oxazoline as a white solid in an excellent yield of 70%.

Furthermore, the Burgess reagent is sensitive to moisture and oxygen and is not even stable when it is stored at low temperature.[48] With time the quality of the reagent decreased, and it become less effective. After further analysis, the yield of the oxazoline decreased to 20% when the Burgess reagent was stored in the lab for more than one month at low temperature. In order to improve the yield of the oxazoline, the concentration of the Burgess reagent was increased to 1.5 and then to 2.0 equivalents, in addition to increasing the reaction time. However, the yield of oxazoline was the same and could not be improved.

2.3.3.1.2 Reaction using DAST reagent

Another dehydrating reagent was used in order to improve the yield of the oxazoline formation reaction. DAST reagent was used for cyclodehydrative conversion of β-hydroxy amide to oxazoline at low temperature.[45,46] In fact, the β -hydroxy amide **(6)** was treated with DAST reagent in anhydrous DCM at -78 ^{0}C for 1 h, followed by the addition of anhydrous K_2CO_3 at the same temperature (**Scheme 9**).[49]



6 **7**

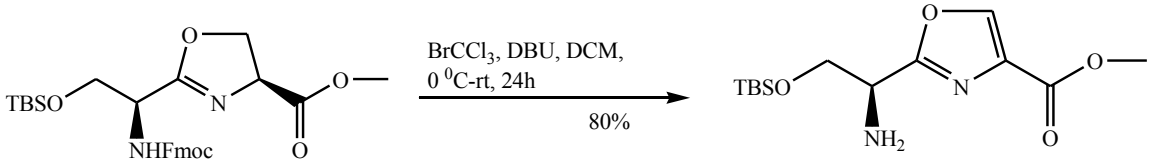
Scheme 9: Synthesis of Fmoc-oxazoline-OMe using DAST reagent.

Then the reaction was allowed to warm to room temperature. Based on the TLC, disappearance of the starting material, the formation of oxazoline was observed and extraction with $NaHCO_3$ was done. The organic layer was dried over $MgSO_4$, filtered and concentrated *in vacuo*. The crude product was subjected to dry column chromatography on SiO_2 using a hexane and ethyl acetate mixture to achieve the desired oxazoline **(7)** in an excellent yield. The pure oxazoline **(7)** was obtained as a white solid in 80% yield, and its structure was confirmed by ^{1}H NMR and ^{13}C NMR. As a result, the DAST reagent offers many advantages over the Burgess reagent, in that it is more stable than the latter reagent. Furthermore, the DAST reagent gave a higher yield of the product, oxazoline, than the Burgess reagent.

2.3.3.2 Oxazole formation

In the method established by Williams and co-workers, the oxidation reaction uses bromotrichloromethane ($BrCCl_3$) and 1,8-Diazabicyclo[5.4.0]undec-7-ene (DBU), **Scheme 10**.[50,51] The reaction was achieved to give the oxazole in 80% yield, but the presence of base, DBU, caused cleavage of the Fmoc group. As a result, not only was the oxidation of oxazoline **(7)** to desired oxazole achieved using the DBU/$BrCCl_3$ oxidation system, but the Fmoc protecting group was removed, giving H_2N-oxazole-OMe **(8)** as a final product. To further explain, the reaction using $BrCCl_3$ and DBU is incompatible with the presence of Fmoc protecting group because the Fmoc group can be cleaved with

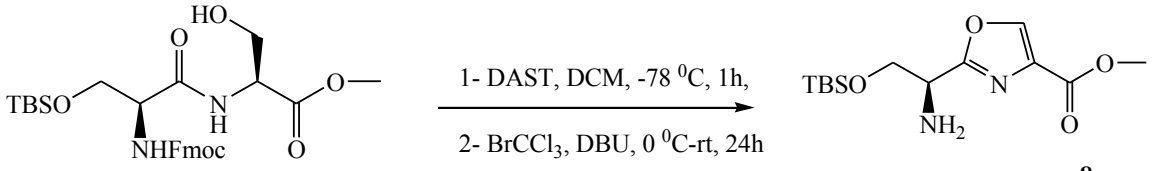
50% DBU in 5 min.[52] Therefore, cleaving the Fmoc group during this process was beneficial because it saved having to cleave the Fmoc group from the Fmoc-oxazole-OMe, meaning it is a one-pot deprotection-oxidation reaction and that was confirmed by ^{1}H NMR (see Appendix 23).



7 **8**

Scheme 10: Synthesis of H_2N-oxazole-OMe.

Synthesis of oxazole can also be achieved in a one-pot process using Fmoc-Ser(O-TBS)-Ser-OMe **(6)** as a starting material. The synthesis of oxazole by one-pot cyclodehydration-dehydrogenation reaction from the dipeptide was obtained using DAST reagent and DBU/$BrCCl_3$ (**Scheme 11**).[49,53]

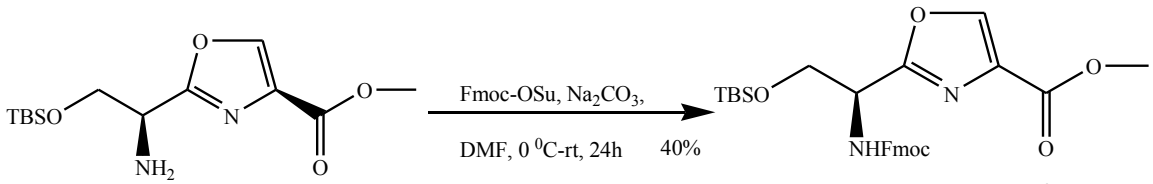


6 **8**

Scheme 11: One-pot synthesis of H_2N-oxazole-OMe from the dipeptide.

First, the dehydration reaction with the DAST reagent was initiated at -78 ^{0}C for 1 h. Followed by dehydrogenation reaction, the addition of DBU was done at -40 ^{0}C and $BrCCl_3$ at 0 ^{0}C, and the reaction was stirred overnight at room temperature. As mentioned earlier, the oxidation using DBU/$BrCCl_3$ was achieved smoothly, and Fmoc group was also removed, resulting in H_2N-oxazole-OMe **(8)** in % 70 yield.

Because the fully protected monooxazole namely Fmoc-oxazole-OMe needed to be synthesized, an effort was made to look for alternative methods to form this compound. Subsequent treatment of the amine, H_2N-oxazole-OMe **(8)**, with Fmoc-OSu in DMF afforded the fully protected monooxazole **(9)** (**Scheme 12**).[54] The reaction was run overnight and then subjected to column chromatography to provide the Fmoc-oxazole-OMe **(9)**; however, this resulted in only 40% yield. The structure was confirmed via ^1H, and ^{13}C NMR spectra (see Appendices 24-28). Hence, this route had more steps and a lower the overall synthesis yield, an effort was made to look for alternative methods to oxidize Fmoc-oxazoline-OMe **(7)** to Fmoc-oxazole-OMe **(9)** without affecting the Fmoc group.

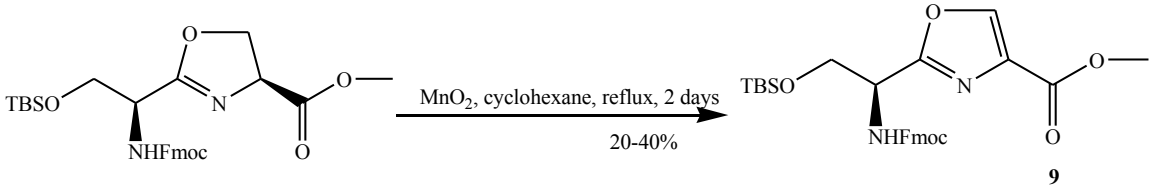


8 **9**

Scheme 12: Protect the amine in H_2N-oxazole-OMe with Fmoc group.

Preliminary studies were done in order to oxidize the intermediate oxazoline **(7)** to oxazole **(9)** using a variety of oxidizing agents and conditions, including nickel peroxide (NiO_2),[55,56] N-bromosuccinimide (NBS) with either peroxide[57] or K_2CO_3,[58] and molecular oxygen with either K_2CO_3[59] or $Cu(OAc)_2$.[60] However, all to no avail as demonstrated by TLC and NMR analysis; in each reaction either the starting material did not react or unidentifiable material resulted.

The conversion of Fmoc-oxazoline-OMe **(7)** to Fmoc-oxazole-OMe **(9)** was also tested by employing an excess amount of activated manganese dioxide (MnO_2). First, the reaction was run in DCM at room temperature overnight using an excess of MnO_2 (10 equivalents), but no cyclization was achieved even when the equivalents of MnO_2 were increased to 20 equivalents and the temperature to 40 ^{0}C.[61,62]Oxazole formation was achieved after changing the solvent to cyclohexane, using 20 equivalents of MnO_2, and increasing the temperature to 80 ^{0}C for 2 days (**Scheme 13**). The work up involved filtering the reaction mixture through celite, washing the celite with EtOAc, and purifying the crude product by column chromatography using EtOAc and hexane.



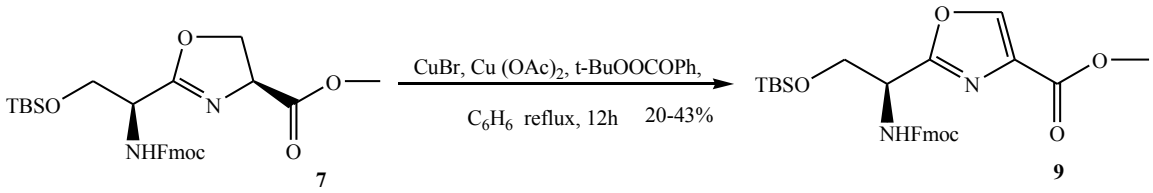
7 **9**

Scheme 13: Synthesis of Fmoc-oxazole-OMe, utilizing MnO_2.

Unfortunately, the oxazoline to oxazole oxidation utilizing MnO_2 in refluxing cyclohexane produced a disappointing result. The yield of the experiment was found to be batch dependent, meaning the yield was different in every reaction even though conditions were left largely unchanged. From the given results of each experiment, the yield ranged from 20% to 40%. The reason for the low yield may have something to do with some of the compound remaining in the celite during the work up because the yield of the crude product was low before the column was done.

The best result for the oxidation of the oxazoline **(7)** to oxazole **(9)** was obtained using the Kharasch-Sosnovsky reaction which involves a cocktail of Cu(I) and Cu(II) salts together with *tert*-butyl perbenzoate. The oxazoline **(7)** was oxidized by employing a mixture of $Cu(OAc)_2$, CuBr, and *tert*-butyl perbenzoate in refluxing benzene for 12 h (**Scheme 14**).[57] The mixture was cooled to room temperature, diluted with ethyl acetate and washed with 10% NH_4OH in order to remove the copper salts. This ethyl acetate

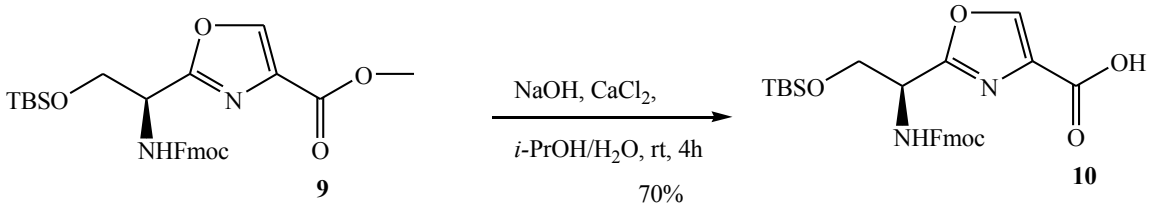
washing process was repeated three times. The combined organic layers were dried over $MgSO_4$ and the solvents were evaporated *in vacuo*. The crude product was purified by column chromatography on SiO_2 and using an EtOAc/hexane system to provide the Fmoc-oxazole-OMe **(9)** as a pale yellow solid in 20-43 % yield. In addition, the reaction time was increased to 24h to see if that could improve the yield of oxazole, but the yield stayed the same.



Scheme 14: Synthesis of Fmoc-oxazole-OMe, utilizing CuBr, $Cu(OAc)_2$.

2.3.4 Acid deprotection using NaOH and $CaCl_2$

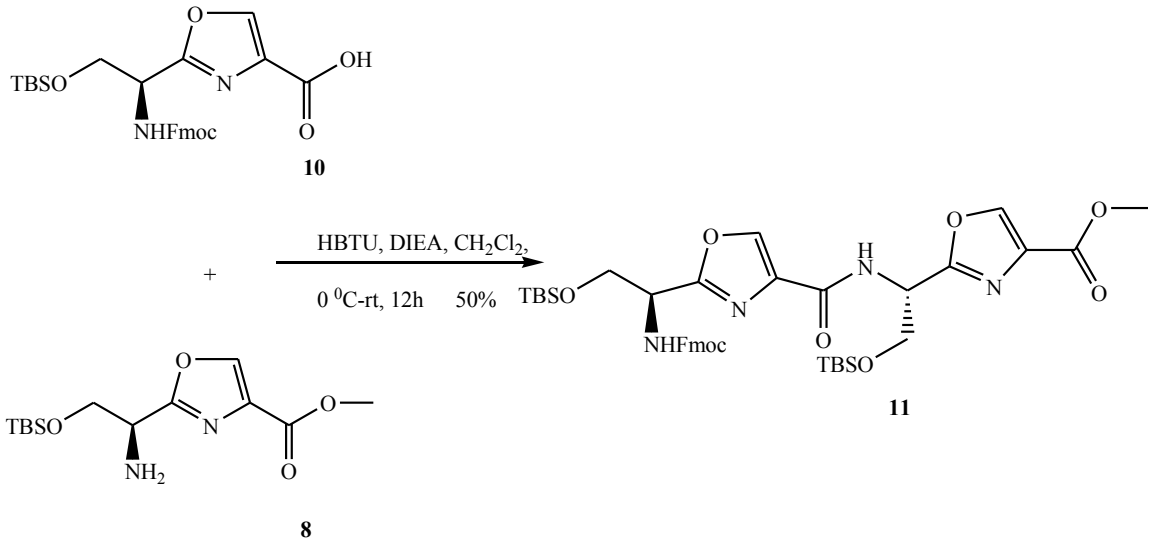
Hydrolysis of the methyl ester protecting group from the monooxazole, Fmoc-oxazole-OMe **(9)**, was achieved by utilizing NaOH and $CaCl_2$ in *i*-PrOH/H_2O (**Scheme 15**).[29] This was done by dissolving the protected monooxazole in *i*-PrOH/H_2O to a 0.1 M concentration with NaOH and $CaCl_2$. The reaction was monitored by TLC, and within 4 h the reaction was completed. The crude free acid Fmoc-oxazole-COOH **(10)** was obtained as a white solid in 70 % yield that was used in the next reaction without further purification. The structure of free acid was confirmed by ^{1}H NMR (see Appendix 29).



Scheme 15: Synthesis of Fmoc-oxazole-COOH.

2.3.5 Synthesis of dioxazole

Coupling of the free acid Fmoc-oxazole-COOH **(10)** and free amine H_2N-oxazole-OMe **(8)** using the coupling reagent HBTU gave the dioxazole amide **(11)**. During this process the free acid **(10)** was dissolved in DMC to a 0.1 M concentration, and HBTU was added at room temperature. After the mixture was cooled to 0 ^{0}C, the base, DIEA, was added. The free amine **(8)** was added last, and the reaction was stirred for 1 h at 0 ^{0}C and then stirred at room temperature for 12h (**Scheme 16**). The work up was done via an acid-base extraction, and the pure dioxazole **(11)** was obtained, after column chromatography using EtOAc and hexane, as a white solid in 50% yield. The structure of pure dioxazole was confirmed by ^{1}H NMR and ^{13}C NMR (see Appendices 30-33).



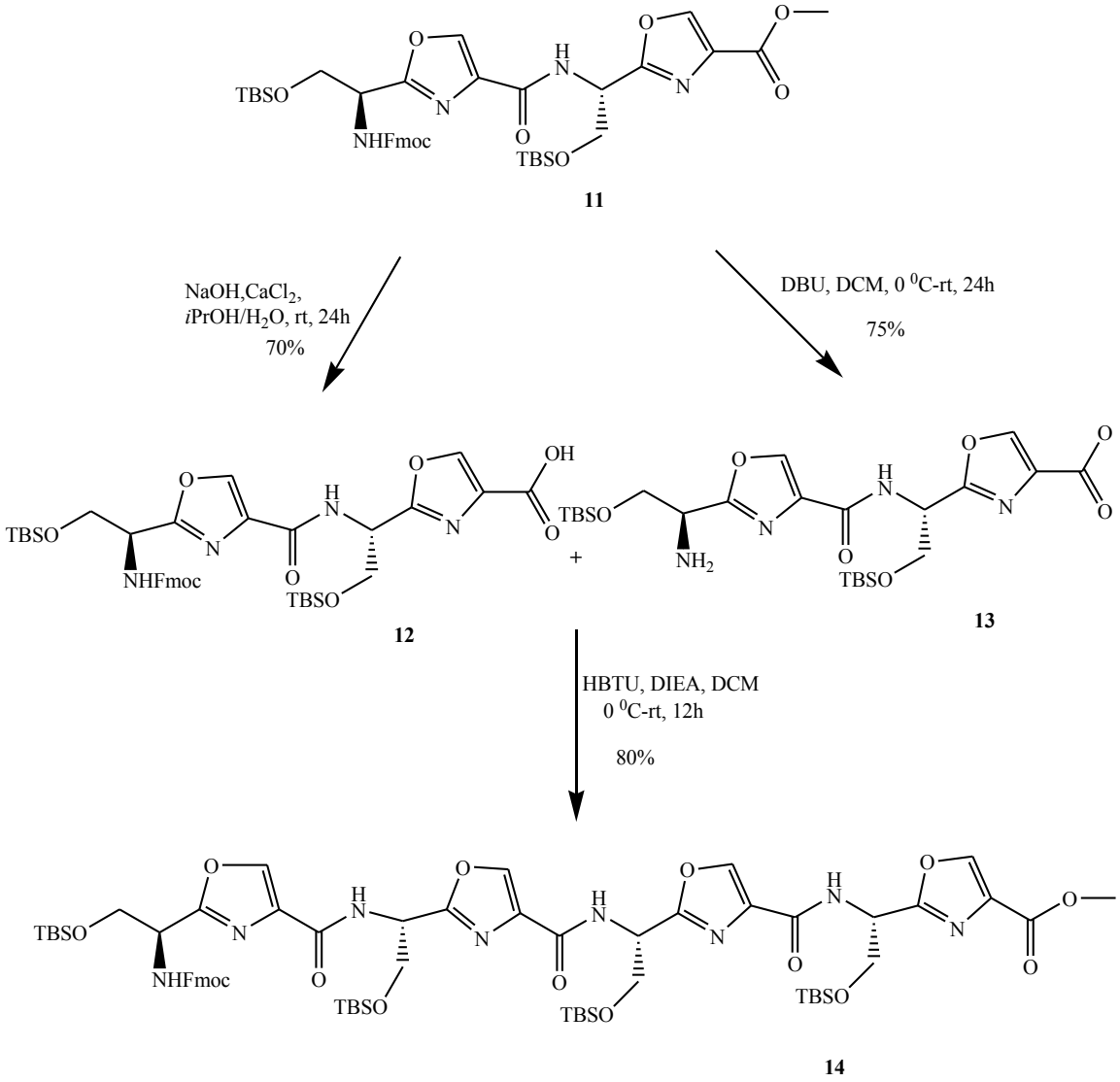
Scheme 16: Synthesis of the dioxazole.

When the reaction time was increased from 12 h to 2 days, the yield was decreased to 35 %. The low yield of the reaction occurred because some of the Fmoc group was cleaved. The presence of the base, DIEA, could have slowly cleaved some the Fmoc group over the 2 day period.[52]

2.3.6 The first route to generate the tetraoxazole using two dioxazoles

The dioxazole **(11)** was divided into two portions, one portion to cleave the methyl ester group, and another to remove the Fmoc group as shown in the **Scheme 17**. The hydrolysis of the methyl ester group was achieved by utilizing NaOH and $CaCl_2$ in *i*-PrOH/H_2O. The crude free acid Fmoc-dioxazole-COOH **(12)**, a white solid, was prepared in 70% yield and was used in the next coupling reaction without further purification. The structure was confirmed via ^{1}H NMR (see Appendix 34). In the second portion of the synthesis, the Fmoc group was removed from the dioxazole **(11)** using the base, DBU, in DCM. This process provided the crude free amine **(13)** in 75% yield that was used in the

next step without further purification, and the structure was confirmed via ^{1}H NMR (see Appendix 35).



Scheme 17: The first route to generate the tetraoxazole.

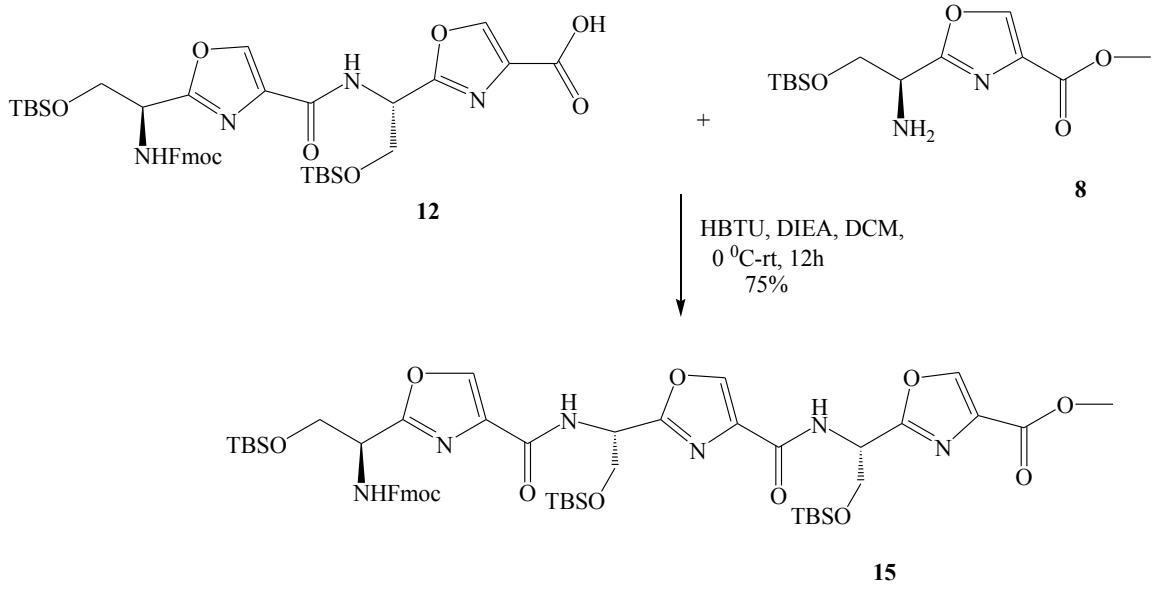
Synthesis of the tetraoxazole **(14)** using a convergent approach involved the coupling of the free acid dioxazole **(12)** and the free amine dioxazole **(13)**. To generate the tetraoxazole **(14)**, the free acid (Fmoc-dioxazole-COOH) **(12)**, and the coupling reagent HBTU were dissolved in DCM at a 0.1 M concentration. The base, DIEA, was then

added to the solution at 0 ^{0}C, followed by the free amine H_2N-dioxazole-COOH **(13)** (**Scheme 17**). The reaction mixture was left to stir at room temperature for 12 h. Upon completion, confirmed by TLC, the crude tetraoxazole was isolated using an acid-base extraction. The pure tetraoxazole **(14)** was eluted from a SiO_2 column using an EtOAc/hexane solvent system yielding a white solid in 80% yield. The structure was confirmed via ^{1}H NMR and ^{13}C NMR (see Appendices 36-40).

2.3.7 The second route to generate the tetraoxazole

2.3.7.1 Synthesis of trioxazole

Because of the low yield of the oxidation step to form Fmoc-oxazole-OMe **(9)**, which is the precursor to form the dioxazole **(11)**, an alternative route to form the tetraoxazole **(14)** was investigated. Instead of using a convergent approach that divides the dioxazole **(11)** into two portions to form the tetraoxazole **(14)** mentioned above, a stepwise approach was developed in order to improve the yield. During the former approach, the methyl ester group was hydrolyzed to form the free acid dioxazole **(12)** and was subsequently coupled with the monooxazole, H_2N-oxazole-OMe **(8)**, to form trioxazole **(15)**. On other worlds, the methyl ester group was cleaved from the dioxazole **(11)** to form the free acid using NaOH and $CaCl_2$ in *i*-PrOH/H_2O. The free acid **(12)** was obtained as a white solid in 92% yield and used in the next coupling reaction without further purification. The free acid **(12)** was coupled with the free amine monooxazole, H_2N-oxazole-OMe **(8)**, to form the trioxazole **(15)**. The coupling reaction was carried out as previously described, using HBTU as a coupling reagent and DIEA as a base in DCM (**Scheme 18**). The work up was achieved by an acid-base extraction, and the crude product was purified by trituration with hot hexane to give the trioxazole in 75% yield. The pure trioxazole was characterized with ^{1}H NMR and ^{13}C NMR (see Appendices 41-42).

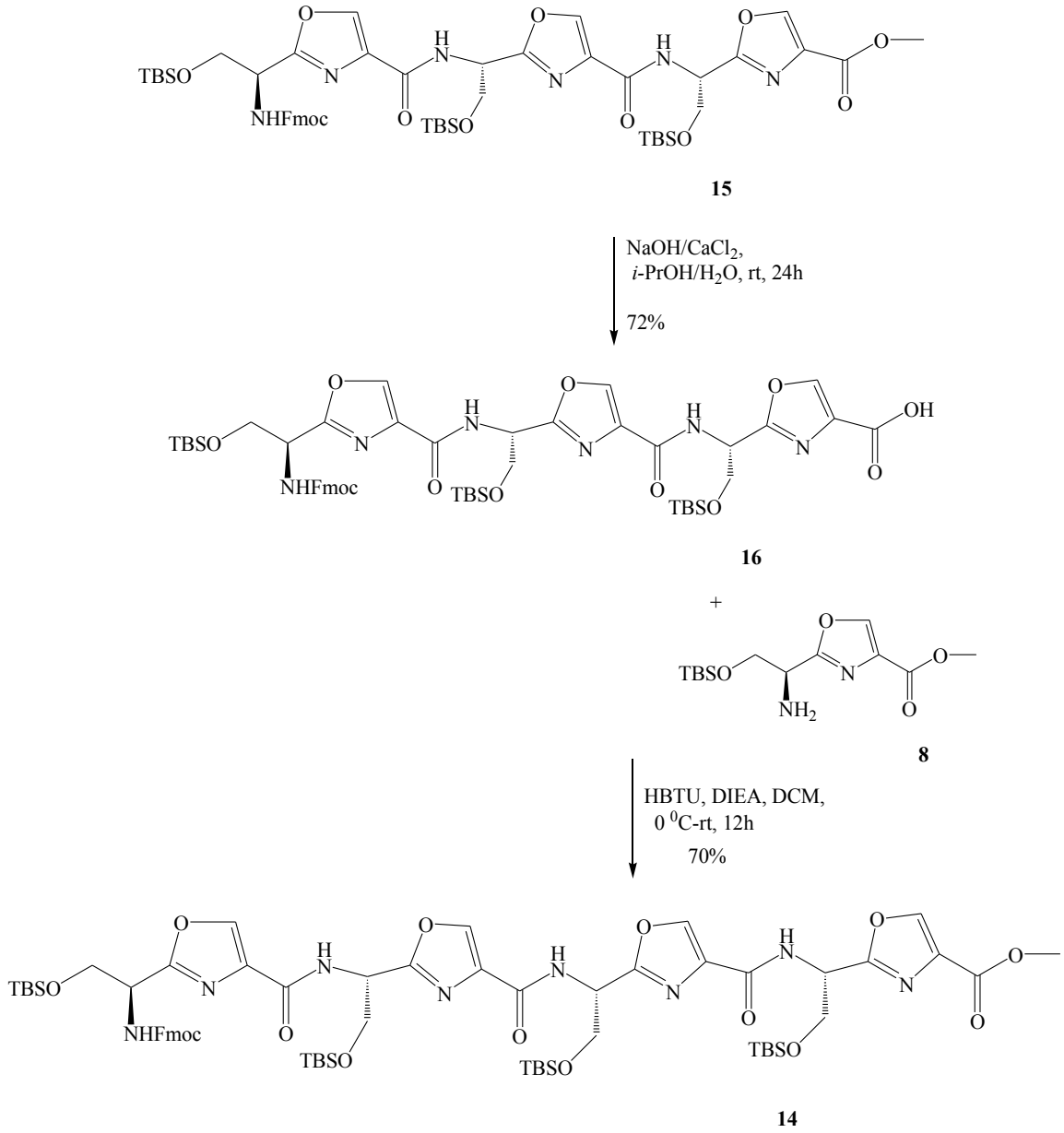


Scheme 18: Synthesis of trioxazole.

2.3.7.2 Synthesis of the linear tetraoxazole

The methyl ester group was cleaved from the trioxazole **(15)** with NaOH and $CaCl_2$ in *i*-PrOH/H_2O as mentioned earlier (**Scheme 19**). The crude free acid, Fmoc-trioxazole-COOH **(16)**, was obtained as a white solid in 72% yield and used in the next reaction without further purification. The structure was characterized with ^{1}H NMR and ^{13}C NMR (see Appendix 43).

The tetraoxazole **(14)** was synthesized using the free acid, Fmoc-trioxazole-COOH **(16)**, the free amine, H_2N-oxazole-OMe **(8)**, combined with the coupling reagent HBTU, and the base, DIEA, (**Scheme 19**). The reaction was stirred for 12 h at room temperature, and the crude tetraoxazole was isolated using an acid-base extraction followed by purification by column chromatography. The pure tetraoxazole **(14)** eluted with 60:40 EtOAc/hexane, was a white solid obtained in 70% yield. The structure and the purity were confirmed by ^{1}H NMR and ^{13}C NMR. This stepwise approach gave a slightly higher yield than the convergent approach.

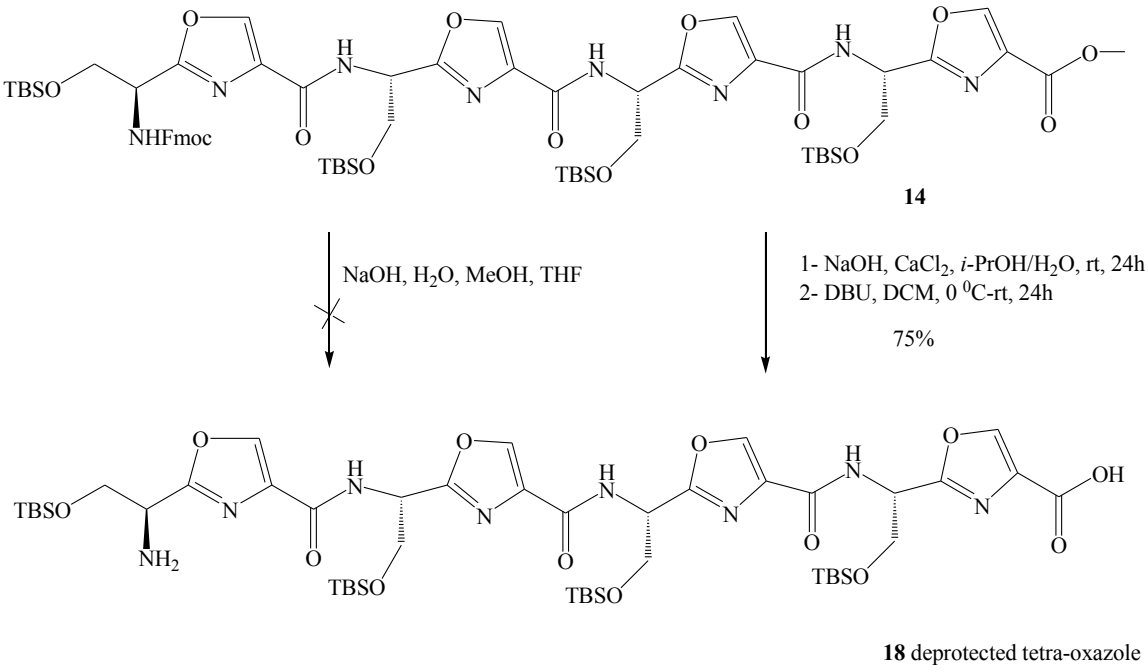


Scheme 19: The second route to generate the tetraoxazole.

2.3.8 Deprotection of tetraoxazole

Two methods were used to provide the doubly deprotected tetraoxazole **(18)**. The first method involved deprotecting the methyl ester group as well as the Fmoc group of the tetraoxazole **(14)** under basic conditions in one step since both of them are base labile.[63] For this method, 0.2 mmol of tetraoxazole dissolved in 1.3 ml of MeOH/THF (1:1 v/v) was added to 1.3 ml of 0.15 M NaOH. The mixture stirred at room temperature overnight, and the organic solvents were evaporated. The residue was diluted with water and acidified with 0.5 N $NaHSO_4$. The mixture was then extracted with DCM, dried over $MgSO_4$, filtered, and concentrated. The solid that was obtained was analyzed by NMR and was not the desired product. It is plausible that the desired compound remained in the aqueous phase due to its polar nature when the work up was completed.

The second method that was used to give the deprotected tetraoxazole **(18)** involved a step-wise approach. In this method, standard acid and amine deprotection conditions were applied respectively (**Scheme 20**).



Scheme 20: Synthesis of the deprotection tetraoxazole.

First, the methyl ester group was cleaved using NaOH and $CaCl_2$ in *i*-PrOH/H_2O to generate free acid Fmoc-tetraoxazole-COOH **(17)**. The free acid was obtained after 24 h of reaction time in 75% yield, and was taken on to the next deprotection step without further purification. Next, the Fmoc group was removed utilizing DBU in DCM to provide the doubly deprotected tetraoxazole **(18)**. The crude doubly deprotected tetraoxazole was analyzed via ^{1}H NMR (see Appendix 44), and an impurity, dibenzofulvene, was generated from Fmoc cleavage. Therefore, the first method did not give the desired product, and the second method worked in 75% yield.

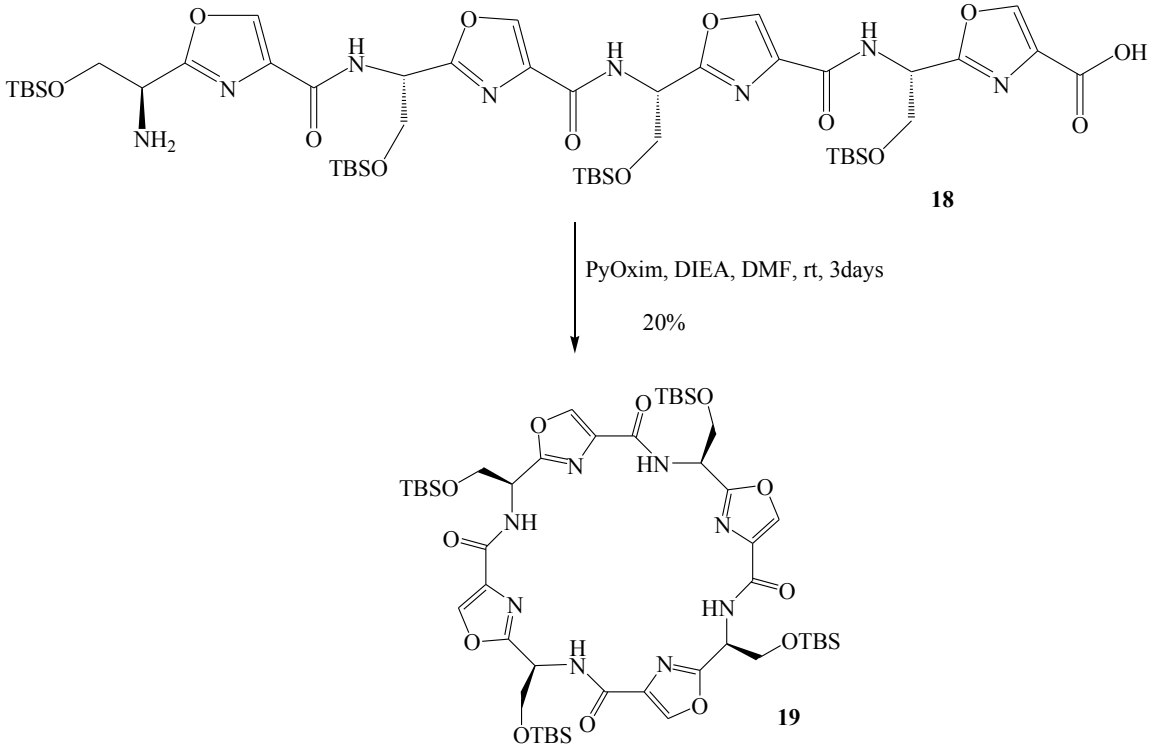
2.3.9 Macrocyclization to generate tetraoxazole macrocyclic peptide

Cyclization reactions are totally different from coupling reactions that provide di-, tri-, and tetraoxazole because the cyclization reactions are run under very dilute conditions (<0.01 M). To explain, cyclization reactions run at a concentration of 0.001 M, whereas the coupling reactions that were previously described occurred at 0.1 M. The concentration of the cyclization reactions is critical in order to prevent intermolecular side reaction since macrocyclization relies on intramolecular ring closure. These dilute conditions help the peptide to generate a macrocyclic peptide, so it provides intramolecular reaction.[27]

Thus, the doubly deprotected linear precursor H_2N-tetraoxazole-COOH **(18)** was allowed to react under a very diluted concentration using coupling reagent *O*-[(1-cyano-2-ethoxy-2-oxoethylidene)amino]-oxytri(pyrrolidin-1-yl) phosphonium hexafluoro-phosphate (PyOxim) and base, DIEA, in DMF. The most potent phosphonium salt nowadays is PyOxim that has an excellent solubility in most organic solvents like DMF. This salt gives high coupling efficiency not only in peptide coupling but also in cyclic peptide synthesis.[64] In contrast to HBTU, PyOxim is stable in solution under inert atmosphere for 2 days. Moreover, since HBTU is such a reactive coupling reagent that leads to side

reactions, usually during a slow coupling reaction like cyclization, PyOxim is a perfect coupling reagent for the synthesis of cyclic peptides.[64]

In order to generate the tetraoxazole macrocyclic peptide **(19)**, the coupling reagent PyOxim, and DIEA were combined with the deprotected tetraoxazole (**Scheme 21**). The coupling reagent and the base were dissolved in dry DMF to a 0.001 M concentration, and a solution of the deprotected tetraoxazole in DMF was added slowly dropwise to the mixture. The reaction mixture was stirred for 3 days, and the crude macrocyclic peptide was subjected to an acid-base extraction. The crude product was purified by column chromatography using an EtOAc/hexane solvent system to give the macrocyclic peptide in 20% yield that was analyzed by ^{1}H NMR (see Appendix 45). Unfortunately, one or more (uncharacterized) contaminants were clearly visible in the NMR spectrum. Attempts to purify the macrocycle by crystallization in hot hexane were not successful. Hence, a pure macrocyclic compound was not obtained.



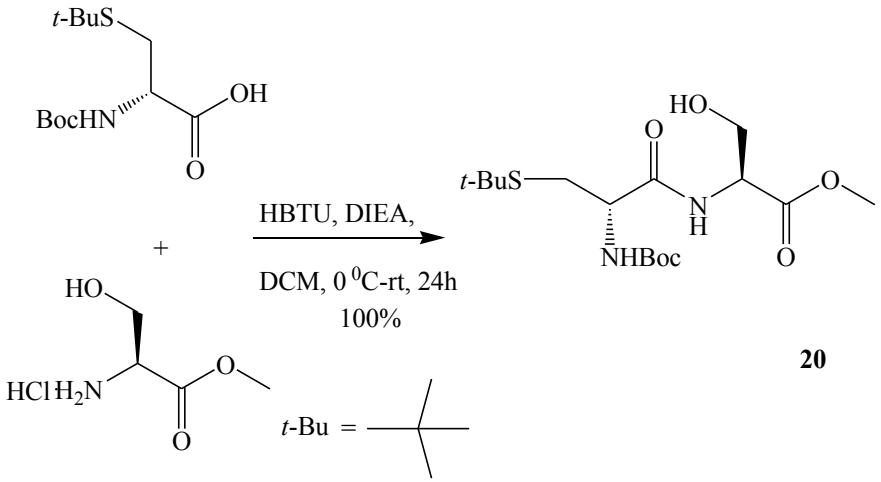
Scheme 21: Synthesis of the tetraoxazole macrocyclic peptide with TBS groups.

2.4 Synthesis of the tetraoxazole macrocyclic peptide with *t*-Bu groups

In summary, the major difficulty in providing the macrocyclic peptide was the low yield of the oxidation reaction, forming the intermediate Fmoc-oxazole-OMe **(9)**, and compounds produced from it. To explain, the Fmoc group was sensitive to the experimental conditions used in the oxidation reaction and was easily removed. To improve the yield of the oxidation step, the starting material amino acid as well as the protecting groups in the amino acid were changed. Boc-Cys(*t*-Bu)-OH was used instead of Fmoc-Ser-OH(OTBS). Once this change was made, an improvement of the oxidation step was noticed. The yield for the oxidation step to get the fully protected monooxazole (Boc-oxazole-OMe) was 48% in three steps whereas the yield for Fmoc-oxazole-OMe was 20% in four steps.

2.4.1 Synthesis of the dipeptide Boc-Cys(*t*-Bu)-Ser-OMe

Synthesis of the second macrocyclic peptide began with the condensation of amino acids Boc-L-Cys(*t*-Bu)-OH and HCl.H_2N-L-Ser-OMe using HBTU as coupling reagent. The free acid Boc-L-Cys(*t*-Bu)-OH, and the HBTU were added into a flask and dissolved in DCM to a 0.1 M concentration (**Scheme 22**). Following this, the base, DIEA, and the free amine as a salt Ser-OMe.HCl were added to the reaction mixture at 0 ^{0}C. The crude dipeptide was purified by crystallization with hot hexane to provide the pure dipeptide Boc-Cys(*t*-Bu)-Ser-OMe **(20)** as a white solid in 100% yield. The pure dipeptide was confirmed by using ^{1}H NMR and ^{13}C NMR (see Appendices 46 and 47).



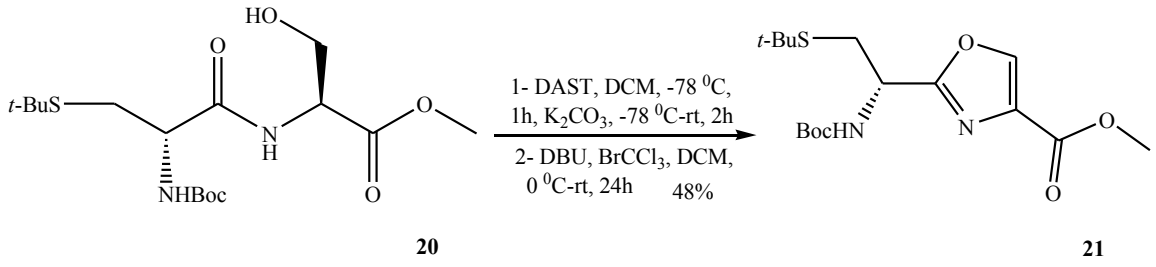
Scheme 22: Synthesis of the dipeptide Boc-Cys(*t*-Bu)-Ser-OMe.

2.4.2 Synthesis of the oxazole ring (Boc-oxazole-OMe)

Cyclodehydration of the β-hydroxy amide was performed by fluorination with the DAST reagent. The dipeptide **(20)** was dissolved in dry DCM to 0.1 M concentration at -78 ^{0}C and under nitrogen gas. DAST reagent was added dropwise over 15 min to the reaction flask and stirred for 2 h. K_2CO_3 was added to the reaction and stirred for 1 h at the same temperature (**Scheme 23**). The reaction was monitored with TLC, and upon completion the crude reaction mixture was diluted with DCM, washed with $NaHCO_3$, dried over $MgSO_4$, filtered, and concentrated. The oxazoline intermediate was used in the next step without further purification.

The oxazoline intermediate was directly subjected to an oxidation reaction using DBU/$BrCCl_3$ (**Scheme 23**). The oxazoline was dissolved in anhydrous DCM at a 0.1 M concentration, and the base, DBU, was added dropwise at 0 ^{0}C under argon gas. After the reaction had been stirred for 5 min, $BrCCl_3$ was added dropwise to the flask. The crude oxazole was purified by column chromatography using an EtOAc/hexane solvent system.

The 3 step process yielded 48% pure Boc-oxazole-OMe **(21)** and its structure was confirmed by using ^{1}H NMR and ^{13}C NMR (see Appendices 48 and 49).

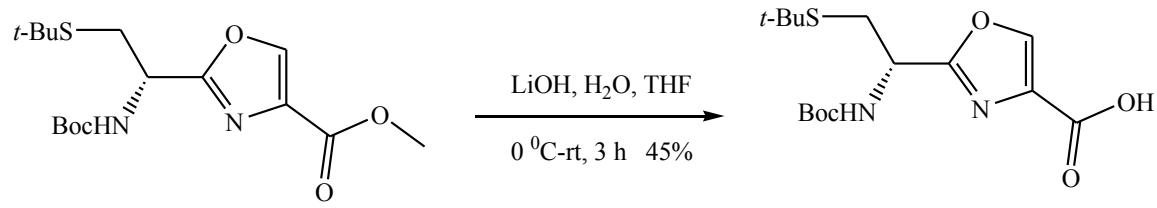


20 **21**

Scheme 23: Synthesis of Boc-oxazole-OMe.

2.4.3 Hydrolysis of the methyl ester group using LiOH

The protected monooxazole, Boc-oxazole-OMe **(21)**, was split into two portions: one to cleave the methyl ester group and another to remove the Boc group. Deprotection of Boc-oxazole-OMe **(21)** to generate the acid Boc-oxazole-COOH **(22)** was carried out under basic conditions. More specifically, the monooxazole was dissolved in THF, to a 0.1 M concentration, and then a solution of LiOH in H_2O was added dropwise to the reaction mixture at 0 ^{0}C (**Scheme 24**).[21] The reaction mixture was stirred and monitored by TLC, up until completion. The free acid Boc-oxazole-COOH **(22)** was obtained as a yellow solid in 45% yield, and the structure was analyzed with ^{1}H NMR and ^{13}C NMR (see Appendices 50 and 51).

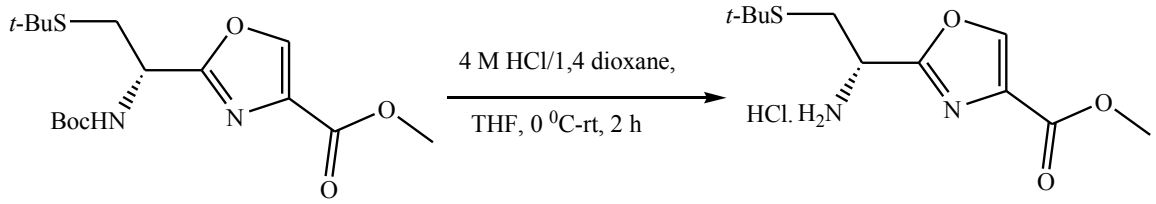


21 **22**

Scheme 24: Synthesis of Boc-oxazole-COOH.

2.4.4 Boc group removal

With Boc-oxazole-OMe **(21)** in hand, cleaving the Boc group was achieved under acidic condition by using 4 M HCl/1,4 dioxane (**Scheme 25**).[39] This process consisted of dissolving the Boc-oxazole-OMe **(21)** in anhydrous THF at 0.3 M concentration followed by the addition of 4 M HCl/1,4 dioxane at 0 ^{0}C and under nitrogen gas. Monitored by TLC up until the disappearance of starting material, the reaction was completed in 2 h at room temperature. The solvents were evaporated *in vacuo*, and the residue was washed with dry ether. Simple decantation was used to provide the HCl salt of amine monooxazole, HCl.H_2N-oxazole-OMe **(23)**, in a quantitative yield. The structure was analyzed with ^{1}H NMR and ^{13}C NMR (see Appendices 52 and 53).

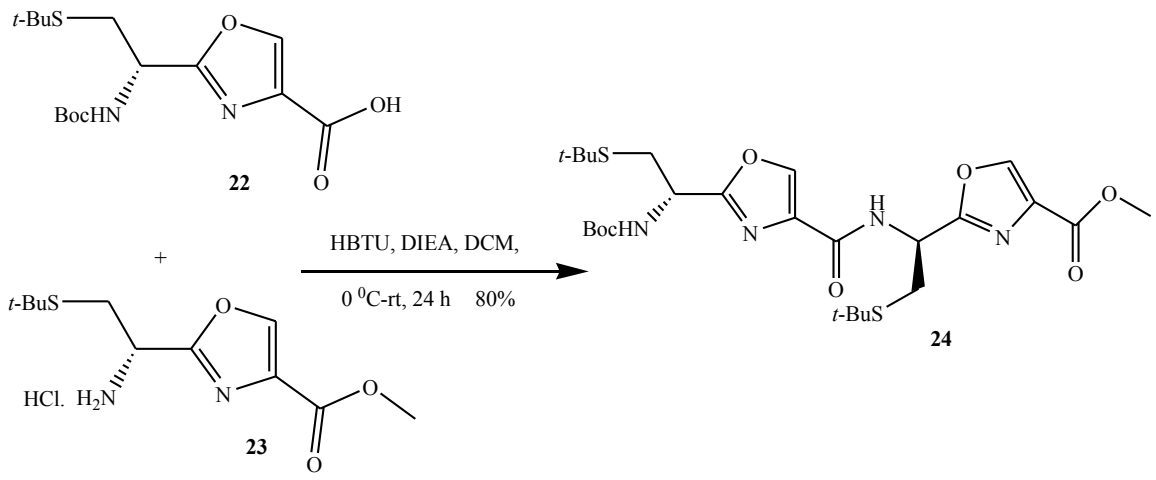


21 **23**

Scheme 25: Synthesis of HCl.H_2N-oxazole-OMe.

2.4.5 Synthesis of dioxazole

Coupling of free acid, Boc-oxazole-OMe **(22)**, with free amine monooxazole, HCl.H_2N-oxazole-OMe **(23)**, provided the dioxazole amide **(24)** under the coupling reaction condition described above. The free acid **(22)** and the coupling reagent HBTU were dissolved in DCM for an overall concentration of 0.1 M. Following this, the base, DIEA, was added slowly at 0 ^{0}C under nitrogen gas, and the free amine **(23)** was added last (**Scheme 26**). The reaction was stirred overnight at room temperature. An acid-base extraction was used to isolate the crude dioxazole. Column chromatography was used to obtain the pure dioxazole **(24)** in 80% yield, and the structure was confirmed by using ^{1}H NMR and ^{13}C NMR (see Appendices 55 and 56).

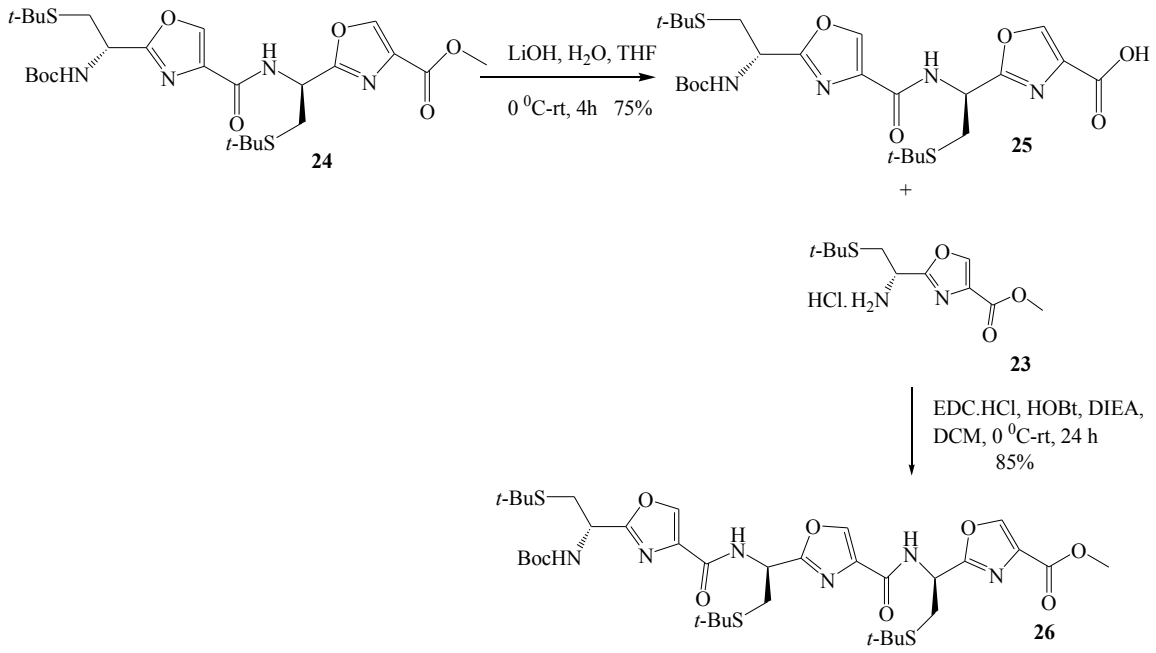


Scheme 26: Synthesis of dioxazole Boc-dioxazole-OMe.

2.4.6 Synthesis of trioxazole (Boc-trioxazole-OMe)

Due to the small quantity of the dioxazole amide **(24)**, instead of dividing the dioxazole amide into two fractions, the methyl ester was cleaved from the dioxazole amide **(24)** to get the acid **(25)** that could be coupled with the amine monooxazole **(23)**. The methyl ester group was removed with LiOH in H_2O/THF, providing the dioxazole-COOH **(25)** as a white solid in 75% yield. The structure was analyzed with ^{1}H NMR and ^{13}C NMR (see Appendices 56 and 57). Following this step, the trioxazole was achieved by utilizing the coupling reagent N-(3-Dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (EDC) with 1-hydroxybenzotriazole (HOBt).[65] EDC is a carbodiimide reagent that is used to activate a carboxylic acid, and it is such a reactive reagent that can cause racemization of the amino acids and produce side reactions during the coupling. Thus, the addition of an additive such as HOBt can minimize racemization and avoid the formation of unreactive side-reactions. Moreover, EDC is highly suitable for use in solution phase peptide synthesis because EDC and its urea by-product are soluble in water and can be removed by aqueous workup.[66]

The free amine HCl.H_2N-oxazole-OMe **(23)** was added to a cold solution of free acid Boc-dioxazole-COOH **(25)** in DCM to a concentration of 0.1 M. Followed by the addition of EDC.HCl and HOBt; the reaction was stirred for 5 min before the base, DIEA, was slowly added (**Scheme 27**). The reaction stirred overnight, and then the crude reaction mixture was subjected to acid-base extraction and further purification by crystallization with hot hexane. The pure protected trioxazole, Boc-trioxazole-OMe **(26)**, was furnished as a solid in 85% yield and the structure was confirmed via ^{1}H NMR and ^{13}C NMR (see Appendices 58 and 59).



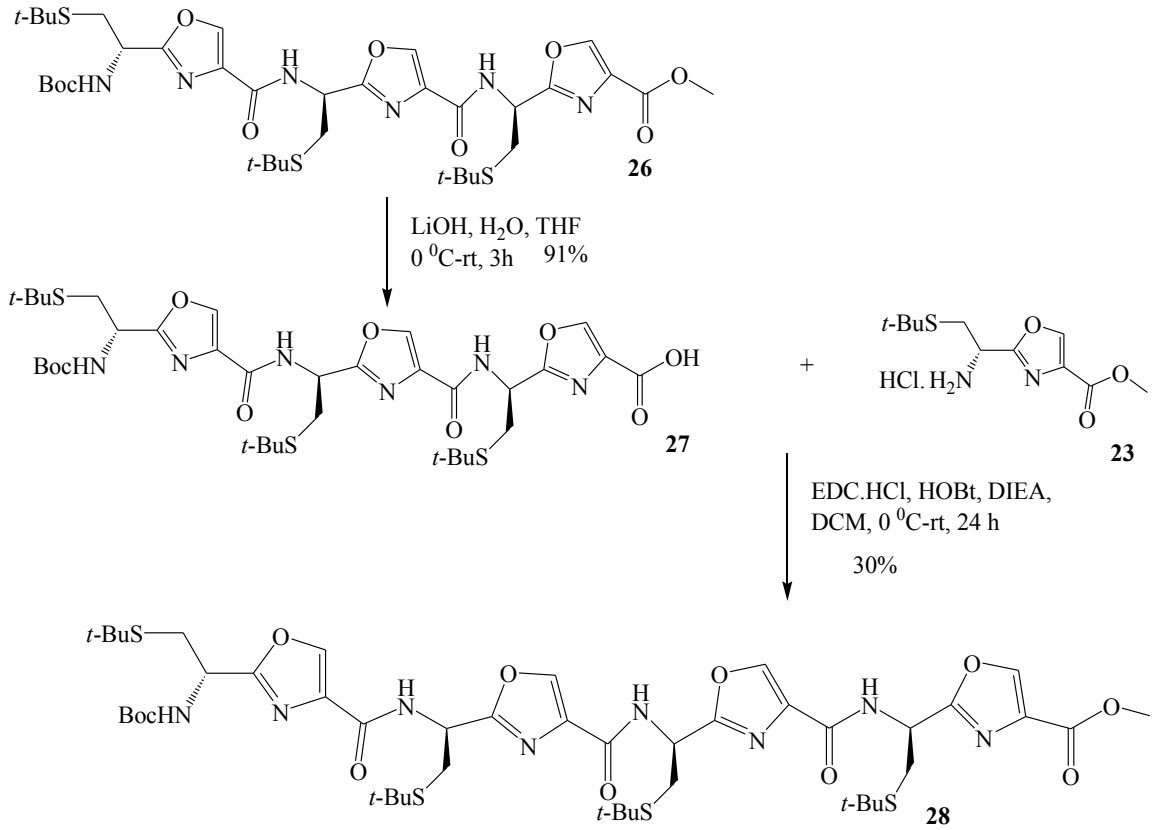
Scheme 27: Synthesis of Boc-trioxazole-OMe.

2.4.7 Synthesis of tetraoxazole (Boc-tetraoxazole-OMe)

Having attained the fully protected trioxazole, the methyl ester protecting group of Boc-trioxazoles-OMe **(26)** was removed in order to prepare the acid **(27)** so that it could be coupled with amine monooxazole **(23)** to obtain the tetraoxazole (**Scheme 28**). Boc-trioxazole-OMe **(26)** was dissolved in THF to a 0.1 M concentration, and LiOH in H_2O was added to the mixture at 0 ^{0}C. The reaction was monitored by TLC up until its

completion, a process lasting 3 h. The reaction mixture was acidified with 1N $NaHSO_4$ and extracted with EtOAc. The organic layer was dried over $MgSO_4$, filtered, and concentrated to provide the free acid in 91% yield. The structure of the pure acid, Boc-trioxazole-COOH **(27)**, was confirmed by using ^1H NMR (see Appendix 60).

Boc-tetraoxazole-OMe **(28)** was synthesized by coupling the acid, Boc-trioxazole-COOH **(27)**, with the free amine, $HCl.H_2N$-oxazole-OMe **(23)**, utilizing EDC.HCl, HOBt, and the base, DIEA, in DCM (0.1 M) at 0 ^0C under argon (**Scheme 28**). The reaction mixture stirred for 1 h at the same temperature then at room temperature overnight. The reaction mixture was then subjected to acid-base extraction, followed by purification via column chromatography. The crude product was first eluted with a DCM/MeOH solvent system from a SiO_2 column to provide the pure compound, however, no separation was obtained. This may have been due to the solubility of the crude product in the DCM/MeOH solvent system being too high. Therefore, the purification of the crude product was repeated, but this time with an EtOAc/hexane solvent system on SiO_2. The pure Boc-tetraoxazole-OMe **(28)** was obtained as a white solid in 30% yield. The structure was confirmed by using ^1H NMR and ^{13}C NMR (see Appendices 61 and 62).

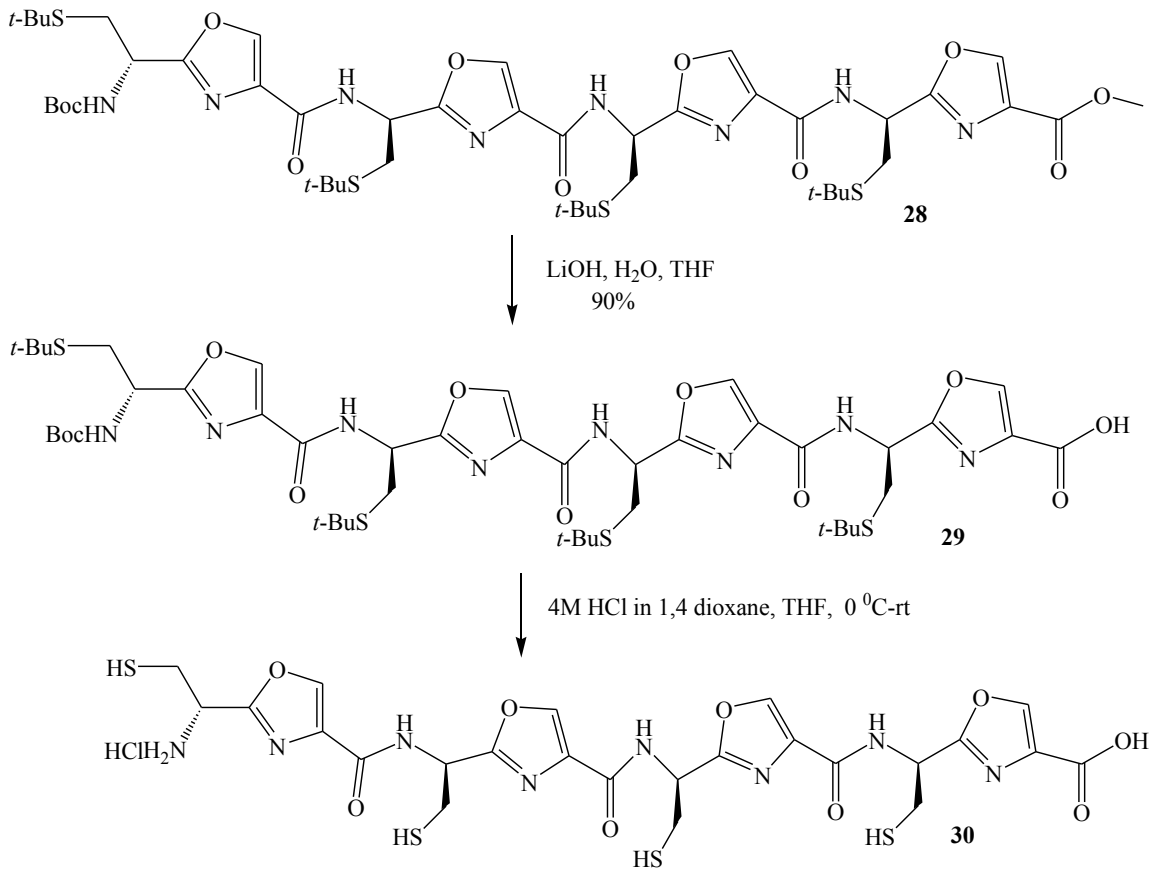


Scheme 28: Synthesis of the tetraoxazole.

2.4.8 Synthesis of the fully deprotected tetraoxazole ($HCl.H_2N$-tetraoxazole-COOH)

The formation of unprotected tetraoxazole involved a stepwise approach by utilizing acid and then amine deprotection conditions (**Scheme**). First, the methyl ester group was removed using LiOH in H_2O/THF to provide the free acid **(29)** in 90% yield that was used in the next deprotection step without further purification. Second, 4 M HCl/1,4 dioxane was used to remove the Boc group in anhydrous THF. After dissolving the Boc-tetraoxazole-COOH **(29)** in THF, 4 M HCl/1,4 dioxane was added to the mixture at 0 ^{0}C under a nitrogen atmosphere. The reaction mixture was stirred for 4 h at room

temperature and then THF was evaporated *in vacuo*. The residue was washed with dry ether and collected by filtration. An analysis of the sample by NMR, revealed the Boc group to be still attached to the amine group. As a result, the deprotection of the Boc group was repeated using the same method except that the reaction was run for 8 h. The work up was done in the same manner as before and the resulting compound **(30)** was analyzed by the ^{1}H NMR (see Appendix 63). Unfortunately, the Boc group and the *t*-Bu group were removed using the longer reaction time. The final step to form the macrocyclization is not expected to work because of the unprotected side chain of the tetraoxazole.



Scheme 29: Synthesis of deprotected tetraoxazole.

2.4.9 Macrocyclization of the tetraoxazole macrocyclic peptide

The macrocyclization reaction was tested using the same condition used in preparing the first macrocyclic peptide **(19)**. The reaction was run with PyOxim as a coupling reagent and DIEA as a base under dilute conditions (0.001 M). The reaction was stirred for 3 days, and the crude mixture was subjected to an acid-base extraction. The crude was purified by column chromatography on SiO_2 using an EtOAc/hexane solvent system. Even though the starting material reacted, according to the TLC results, the anticipated cyclic product was not obtained. Although the reason for the absence of the macrocycle in the reaction mixture is not known, it is possible that partial oxidation of the free sulfhydryl groups (leading to the formation of disulfides) could lead to a higher rigidity of the tetraoxazole, hence impairing the attack of the free N-terminal amine on the activated C-terminal carboxylate. Alternatively, it might be possible that some of the free thiol groups of the tetraoxazole reacted with the PyOxim-activated C-terminal carboxylate to form a thioester (instead of the N-terminal amine group).

Chapter 3- Experimental Methods

3.1 General remarks about reagents and instruments

All reactions were carried out under a nitrogen atmosphere with dry organic solvents, unless otherwise noted. Some of the organic solvents were dried before use such as tetrahydrofuran (THF) and dichloromethane (DCM). THF was distilled over sodium and benzophenone whereas DCM was distilled over calcium chloride. Other organic solvents such as anhydrous dimethylformamide (DMF) were used as they were obtained from commercially available sources (Fisher, and Sigma-Aldrich). Reagents were obtained from commercial sources and were used in the reactions as received without further purification.

All reactions were monitored via thin-layer chromatography (TLC) using TLC plates made of silica gel F-254. A mixture of solvents was also used, primarily ethyl acetate/hexanes or methanol/DCM. The plates were visualized with ultraviolet light at a wavelength of λ = 254 nm and then developed with a ninhydrin solution that was prepared by dissolving 5 g of phosphomolybdic acid hydrate in 95 ml of ethanol.

Column chromatography was performed on two kinds of silica gel (silica gel 60 (70-230 mesh ASTM) and (230–400 mesh ASTM)) that were obtained from Silicycle (Quebec, Canada) and used as the stationary phase.

All evaporations were carried out at 40 ^{0}C under reduced pressure using a rotary evaporator. Melting points were determined with a Melting Point Apparatus in capillary tubes from Kimble Chase.

All compounds were analyzed using a Bruker 500 MHz nuclear magnetic resonance (NMR) spectrometer at room temperature. Proton (^{1}H) NMR and carbon (^{13}C) were recorded in deuterated solvents, and residual undeuterated solvent was used as an internal reference. The chemical shifts are quoted in parts per million (ppm), and all coupling constants are quoted in Hertz. The following abbreviations were used to explain multiplicity of each signal: s = singlet, d = doublet, t = triplet, q = quartet, p = pentet, m =

multiplet, brd = broad, bd = broad doublet, dd = doublet of doublet, and dq = doublet of quartet. ^{13}C NMR was further analyzed by distortionless enhancement by polarization transfer (DEPT) 135 and DEPT 90 in order to identify carbon peaks. DEPT spectra determine the number of hydrogens attached to each carbon in a compound. A DEPT 135 experiment yields CH and CH_3 as positive peaks whereas CH_2 as negative signals. In addition, a DEPT 90 experiment gives only CH peaks and quaternary carbon peaks are disappeared in both DEPT 135 and DEPT 90. Where required, a compound can also be analyzed by two-dimensional NMR such as correlation spectroscopy (COSY) of ^{1}H-^{1}H correlations which identify coupled protons.

3.2 General experimental procedures

3.2.1 Solution phase peptide coupling

Peptide coupling reactions were preformed using different coupling reagents such as HBTU or EDC.HCl with HOBt in solution phase synthesis. The reactions were carried out under a nitrogen atmosphere with anhydrous DCM. The free acid (1.0 equivalent) was weighed in a dry flask then dissolved in dry DCM to give a 0.1 M solution. The coupling reagent (1.2 equivalents of HBTU or 1.2 equivalents of EDC.HCl with 1.2 equivalents of HOBt) was added to the reaction and stirred for 5 min at room temperature. After that, the reaction mixture was cooled to $0\ ^{0}C$ and the base DIEA (2.5 equivalents) was added to the reaction flask. Finally, (1.0 equivalent) of the free amine was added to the reaction mixture at the same temperature. The reaction was stirred overnight while it was allowed to warm up to room temperature. The completion of the reaction was assumed by checking the TLC. The reaction was then worked up with an acidic aqueous solution like 0.5 N $NaHSO_4$ in order to remove any excess of free amine, followed by a wash with saturated $NaHCO_3$ in order to remove the excess of coupling agent(s) and their by-products. The organic layers were collected, washed with H_2O and brine and dried over $MgSO_4$. The filtrate was concentrated *in vacuo* to give the crude and

then column chromatography was performed using an EtOAc/hexane system to give the desired purified peptide.

3.2.2. The deprotection of the Fmoc group

DBU (2.0 equivalents) was added slowly to a solution of the Fmoc protected peptide in DCM (0.1 M) at 0 ^{0}C under argon. The resulting mixture was stirred for 1 h at 0 ^{0}C, and then warmed to room temperature and stirred overnight to ensure complete removal of the Fmoc protecting group. The mixture was diluted with DCM and washed with H_2O, dried over $MgSO_4$ and filtered. The filtrate was concentrated *in vacuo* to give the free amine.

3.2.3 The deprotection of the Boc protecting group

The Boc protected compound was dissolved in dry THF (0.1 M) and the solution was cooled to 0 ^{0}C under N_2 gas. 4 M HCl/1,4 dioxane (33.0 equivalents) were added at the same temperature and stirred for 5 minutes. The ice bath was then removed and stirring continued at room temperature until the reaction was complete as monitored by TLC. The mixture was concentrated *in vacuo* to obtain the free amine as HCl salt. The salt was then washed with dry ether several times in order to remove any remaining solvents and HCl.

3.2.4 The hydrolysis of the methyl ester group

Two different procedures were used to remove the methyl ester group from the protected peptide: the first using NaOH and $CaCl_2$ and the second using LiOH.

3.2.4.1 Cleaving the methyl ester group using NaOH and $CaCl_2$

The methyl ester protected peptide was dissolved in H_2O: *i*-PrOH (3:7, 0.1M), and NaOH (1.2 equivalents) and $CaCl_2$ (2.5 equivalents) were added to the mixture. The reaction was stirred until the TLC showed the disappearance of all starting material. The resulting mixture was acidified with 0.5 N $NaHSO_4$. The aqueous layer was extracted twice with EtOAc, and the organic layer was washed with water and brine, dried over $MgSO_4$ and

filtered. The filtrate was evaporated to give the crude acid that was used in the next reaction without further purification.

3.2.4.2 Cleaving the methyl ester group using LiOH

The protected compound was dissolved in THF for a 0.1 M concentration and the solution was cooled to 0 ^{0}C under a nitrogen atmosphere. LiOH.H_2O (2.0 equivalents) in H_2O (0.1 M) was added dropwise over 15 min at 0 ^{0}C, and the mixture was then stirred and warmed up to room temperature. The progress of the reaction was monitored with TLC up until its completion. At that point, the mixture was evaporated *in vacuo*. The residue was diluted with water, acidified with HCl to pH=3 and extracted twice with EtOAc. The combined organic layer was washed with brine, dried over $MgSO_4$, filtered and concentrated *in vacuo* to yield the free acid.

3.2.5 Macrocyclization

Macrocyclization was performed under diluted condition (0.001 M) using PyOxim (1.2 equivalents) and base DIEA (3.2 equivalents) in anhydrous DMF at room temperature under a nitrogen atmosphere and stirred for 3 days. The solvent was evaporated *in vacuo*, and the residue was then dissolved with DCM. The mixture was washed once each with 1 N $NaHSO_4$, saturated $NaHCO_3$, water, and brine. The organic layer was dried over $MgSO_4$, filtered, and evaporated *in vacuo* to give the crude macrocyclic peptide that was purified by column chromatography on SiO_2.

3.3 Experimental methods for the synthesis of the tetraoxazole macrocyclic peptide with TBS groups

3.3.1 Boc chemistry

3.3.1.1 Synthesis of Boc-Ser-OH(O-TBS) (1)

Boc-L-Ser-OH (1.73 g, 8.43 mmol) was dissolved in DMF (0.2 M, 42.15 ml) and the mixture was cooled to 0 ^{0}C under a nitrogen atmosphere. TBSCl (1.9 g, 12 mmol) and

imidazole (1.77 g, 26.9 mmol) were added to the mixture, which was then stirred for 1 h at the same temperature. Following this step, the mixture was warmed to room temperature and stirred overnight. The mixture was partitioned between Et_2O and H_2O and the aqueous layer was re-extracted twice with ether. The organic layers were combined and dried over $MgSO_4$ and filtered. The filtrate was concentrated *in vacuo* to provide an oily residue that was purified by column chromatography on SiO_2 using an ethyl acetate, hexane, and AcOH system to afford a 50% yield of Boc-Ser-OH(O-TBS) **(1)** as colorless oil (see Appendices 1 and 2). ^{1}H NMR (500 MHz, Chloroform-*d*) δ 5.36 (d, *J* = 8.3 Hz, 1H; NH), 4.40 (brd, 1H; CH), 4.12 (m, 1H; CH_2), 3.85 (dd, *J* = 10.1, 4.0 Hz, 1H; CH_2), 1.48 (s, 9H; $(CH_3)_3$ Boc), 0.90 (s, 9H; $(CH_3)_3CSi(CH_3)_2$), 0.08 (d, *J* = 5.3 Hz, 6H; $(CH_3)_3CSi(CH_3)_2$). ^{13}C NMR (126 MHz, $CDCl_3$) δ 175.09; (CO)OH, 155.64; (CO)O Boc, 80.29; $C(CH_3)$ Boc, 63.41; CH_2OSi, 55.21; $CHCH_2OSi$, 28.31; $(CH_3)_3$ Boc, 25.74; $(CH_3)_3CSi(CH_3)_2$, 18.22; $(CH_3)_3CSi(CH_3)_2$, -5.54 (two peaks); $(CH_3)_3CSi(CH_3)_2$.

3.3.1.2 Synthesis of Boc-Ser(O-TBS)-Ser-OMe (2)

HBTU (1.5 g, 4.1 mmol) was added to a solution of Boc-Ser-OH(O-TBS) **(1)** (1 g, 3.13 mmol) in dry DCM (0.1 M, 30 ml), and then cooled in an ice-water bath to 0 ^{0}C under a nitrogen atmosphere. DIEA (1.72 ml, 9.92 mmol) was then added, and the reaction mixture was stirred for 10 min. $HCl.H_2N$-L-Ser-OMe (0.48 g, 3.13 mmol) was added and the reaction mixture was stirred for 1 h at 0 ^{0}C. Following this, the ice-bath was removed and the reaction mixture was left to stir overnight at room temperature. The mixture was then diluted with DCM and washed once with each 0.5 N $NaHSO_4$, $NaHCO_3$, brine and water. The organic layer was dried over $MgSO_4$, filtered and evaporated using *vacuo* to provide the crude dipeptide which was then purified by crystallization with hot hexane to provide the dipeptide **(2)** as a yellow solid in 60% yield (see Appendices 3 and 4), mp 56-58 ^{0}C. ^{1}H NMR (500 MHz, Chloroform-*d*) δ 7.37 (d, *J* = 7.1 Hz, 1H; NHCO), 5.39 (brd, 1H; NH Boc), 4.67 (brd, 1H; $CHCH_2OSi$), 4.20 (b, 1H; $CHCH_2OH$), 4.05 (dd, *J* = 9.9, 4.0 Hz, 1H; CH_2OSi), 3.96 (brd, 2H; CH_2OH), 3.80 (s, 3H; OCH_3), 3.74 (dd, *J* = 9.9, 6.1 Hz, 1H; CH_2OSi), 1.48 (s, 9H; $(CH_3)_3$ Boc), 0.91 (s, 9H; $(CH_3)_3CSi(CH_3)_2$), 0.10 (s, 6H; $(CH_3)_3CSi(CH_3)_2$). ^{13}C NMR (126 MHz, $CDCl_3$) δ 171.00; (CO)OCH_3, 171.47; (CO)NH, 155.61; (CO)O Boc, 80.42; $C(CH_3)$ Boc, 63.27; CH_2OSi, 63.18; CH_2OH, 55.96;

$\underline{C}HCH_2OSi$, 55.09; $\underline{C}HCH_2OH$, 52.77; $O\underline{C}H_3$, 28.29; $(\underline{C}H_3)_3$ Boc, 25.28; $(\underline{C}H_3)_3CSi(CH_3)_2$, 18.23; $(CH_3)_3\underline{C}Si(CH_3)_2$, -5.54; $(CH_3)_3CSi(\underline{C}H_3)_2$.

3.3.1.3 Cleaving the Boc group with 4 M HCl in dioxane H_2N-Ser-OH-Ser-OMe (3)

4 M of HCl in dioxane (4 ml, 16 mmol) was cooled to 0 ^{0}C in round bottomed flask using an ice-bath under a nitrogen atmosphere. The dimer Boc-Ser-OH(O-TBS)-Ser-OMe **(2)** (0.084 g, 0.2 mmol) was added into the flask in one portion and the ice-bath was removed. The reaction was stirred at room temperature for half an hour, up until the disappearance of the starting material monitored by TLC. The solvent was removed under reduced pressure, and the residue was then washed with dry ether several times. Finally, the solvent was evaporated to give the dimer as H_2N-Ser-OH-Ser-OMe **(3)** in 70% yield (see Appendices 5 and 6). ^{1}H NMR (500 MHz, Deuterium Oxide) δ 4.57 (t, *J* = 4.2 Hz, 1H; $C\underline{H}CH_2OH$), 4.13 – 4.09 (t, *J* = 5.1 Hz, 1H; $C\underline{H}CH_2OH$), 3.95 (dd, *J* = 12.4, 4.0 Hz, 1H; $C\underline{H}_2OH$), 3.88 (dd, *J* = 10.8, 5.2 Hz, 2H; $C\underline{H}_2OH$), 3.80 (dd, *J* = 11.8, 3.8 Hz, 1H; $C\underline{H}_2OH$), 3.68 (s, 3H; $OC\underline{H}_3$), 3.64 (s, 3H). ^{13}C NMR (126 MHz, Deuterium Oxide) δ 171.65; $(\underline{C}O)OCH_3$, 167.87; $(\underline{C}O)NH$, 61.89; $\underline{C}H_2OH$, 60.14; $\underline{C}H_2OH$, 54.99; $\underline{C}HCH_2OH$, 54.53; $\underline{C}HCH_2OH$, 53.13; $O\underline{C}H_3$.

3.3.2 Fmoc chemistry

3.3.2.1 Synthesis of Ser-OH(O-TBS) (4)

Imidazole (1.37 g, 20 mmol) and TBSCl (1.65 g, 11 mmol) were added to a solution of L-Ser-OH (1.06 g, 10 mmol) in DMF (0.1M, 100 ml) and stirred overnight. The solvents were evaporated under reduced pressure to provide an oily residue and finally, the crude was purified by crystallization using hexane and water (1:1) to afford Ser-OH(O-TBS) **(4)** as a white solid in 80% yield (see Appendices 7 and 8). ^{1}H NMR (500 MHz, Methanol-d_4) δ 4.10 – 4.00 (m, 2H; $C\underline{H}_2$), 3.66 (dd, *J* = 5.9, 3.7 Hz, 1H; $C\underline{H}$), 0.95 (s, 9H;

($\underline{CH_3})_3CSi(CH_3)_2$), 0.14 (d, *J* = 2.4 Hz, 6H; $(CH_3)_3CSi(\underline{CH_3})_2$). ^{13}C NMR (126 MHz, $CDCl_3$) δ 168.76; (<u>C</u>O)OH, 60.57; CH<u>C</u>H_2OSi, 58.85; <u>C</u>HCH_2OSi, 23.40; ($\underline{C}H_3)_3CSi(CH_3)_2$, 16.32; $(CH_3)_3\underline{C}Si(CH_3)_2$, -8.33; $(CH_3)_3CSi(\underline{C}H_3)_2$.

3.3.2.2 Synthesis of Fmoc-Ser-OH(O-TBS) (5)

Imidazole (1.09g, 16mmol) was added to a solution of Fmoc-L-Serine (1.05 g, 3.2 mmol) in DMF (6.4 ml) at room temperature. The solution was cooled to 0 ^{0}C and TBSCl (1.21 g, 8 mmol) was added and stirred for 3 h. Following this step, the reaction mixture was warmed to room temperature and stirred overnight. The solution was diluted with EtOAc to 200ml and washed with NH_4Cl (3×40 ml) and H_2O (40 ml), then dried over $MgSO_4$ and concentrated *in vacuo* to provide a residue. Purification was then performed by using column chromatography on silica gel 40-63μm, eluting with 5% methanol, 94% dichloromethane and 1% AcOH to provide Fmoc-Ser-OH(O-TBS) **(5)** as a white solid in 60% yield (see Appendices 9-12), mp 105-106 ^{0}C. ^{1}H NMR (500 MHz, Chloroform-*d*) δ 7.80 (d, *J* = 7.2 Hz, 2H; 2x Fmoc <u>H</u> Ar), 7.64 (t, *J* = 8.1 Hz, 2H; 2x Fmoc <u>H</u> Ar), 7.43 (t, *J* = 7.3 Hz, 2H; 2x Fmoc <u>H</u> Ar), 7.34 (t, *J* = 7.2 Hz, 2H; 2x Fmoc <u>H</u> Ar), 5.65 (d, *J* = 7.2 Hz, 1H; N<u>H</u>), 4.50 (brd, 1H; αC<u>H</u>), 4.47 – 4.37 (m, 2H; Fmoc C<u>H</u>$_2$), 4.28 (t, *J* = 7.5 Hz, 1H; Fmoc C<u>H</u>), 4.18 (dd, *J* = 9.7, 2.3 Hz, 1H; β C<u>H</u>$_2$), 3.90 (dd, *J* = 9.6, 4.2 Hz, 1H; β'C<u>H</u>$_2$), 0.93 (s, 9H; ($\underline{CH_3})_3CSi(CH_3)_2$), 0.11 (d, *J* = 4.6 Hz, 6H; $(CH_3)_3CSi(\underline{CH_3})_2$). ^{13}C NMR (126 MHz, $CDCl_3$) δ 174.70; (<u>C</u>O)OH, 156.03; O<u>C</u>ONH, [143.67, 141.31, 127.75, 127.08, 125.10, 120.01]; <u>C</u>H Fmoc Ar, 67.34; CH<u>C</u>H_2OSi, 63.25; Fmoc <u>C</u>H_2, 55.43; <u>C</u>HCH_2OSi, 47.10; Fmoc <u>C</u>H, 25.74; ($\underline{C}H_3)_3CSi(CH_3)_2$, 18.22; $(CH_3)_3\underline{C}Si(CH_3)_2$, -5.53; $(CH_3)_3CSi(\underline{C}H_3)_2$.

3.3.2.3 Synthesis of Fmoc-Ser(O-TBS)-Ser-OMe (6)

HBTU (9.15 g, 24.12 mmol) was used to treat a solution of Fmoc-Ser-OH(O-TBS) **(5)** (8.4 g, 20.16 mmol) in DCM (200 ml, 0.1 M) at room temperature. The flask was then placed under a nitrogen atmosphere and the mixture was cooled to 0 ^{0}C. Following this step, DIEA (11.20 ml, 64.32 mmol) was added to the mixture and stirred for 5 min. H-

Ser-L-OMe.HCl (3.14 g, 20.1 mmol) was then added and the resulting mixture was stirred at room temperature overnight. Upon the completion of the reaction, the solvent was evaporated *in vacuo*. The resulting residue was diluted with ethyl acetate, washed with 0.5 N $NaHSO_4$ and the aqueous layer was re-extracted with ethyl acetate. Next, the organic layers were collected and washed with $NaHCO_3$, followed by H_2O and brine. The organic layer was then dried over anhydrous magnesium sulfate, filtered and concentrated *in vacuo*. Finally, the crude dipeptide was purified by crystallization with an ethyl acetate and hexane system to provide the dipeptide **(6)** (1.7 g, 3.25 mmol) as a white solid in 80% yield (see Appendices 13-17), mp 125-126 ^{0}C. ^{1}H NMR (500 MHz, Chloroform-*d*) δ 7.79 (d, *J* = 7.5 Hz, 2H; 2x Fmoc H Ar), 7.62 (t, *J* = 6.0 Hz, 2H; 2x Fmoc H Ar), 7.46 (brd, 1H; NH amide), 7.43 (t, *J* = 7.5 Hz, 2H; 2x Fmoc H Ar), 7.34 (t, *J* = 7.4 Hz, 2H; 2x Fmoc H Ar), 5.73 (brd, 1H; NH Fmoc), 4.69 (m, 1H; NHCHCH$_2$OH), 4.43 (d, *J* = 6.7 Hz, 2H; Fmoc CH$_2$), 4.30 (brd, 1H; NHCHCH$_2$OSi), 4.26 (t, *J* = 6.7 Hz, 1H; Fmoc CH), 4.08 (dd, *J* = 9.8, 4.1 Hz, 1H; NHCHCH$_2$OSi), 4.04 – 3.94 (m, 2H; CHCH$_2$OH), 3.81 (s, 3H; OCH$_3$), 3.74 (dd, *J* = 9.8, 7.1 Hz, 1H; NHCHCH$_2$OSi), 0.94 (s, 9H; (CH$_3$)$_3$CSi(CH$_3$)$_2$), 0.13 (s, 6H; (CH$_3$)$_3$CSi(CH$_3$)$_2$). ^{13}C NMR (126 MHz, $CDCl_3$) δ 170.63; (CO)OCH$_3$, 170.40; CONH amide, 156.15; OCONH, [143.63, 141.31]; C Fmoc Ar, 127.75, 127.08, 125.11, 120.01]; CH Fmoc Ar, 67.33; Fmoc CH$_2$, 63.18; CHCH$_2$OSi, CHCH$_2$OH, 55.99; CHCH$_2$OSi, 55.01; CHCH$_2$OH, 52.77; OCH$_3$, 47.10; Fmoc CH, 25.80; (CH$_3$)$_3$CSi(CH$_3$)$_2$, 18.23; (CH$_3$)$_3$CSi(CH$_3$)$_2$, -5.54; (CH$_3$)$_3$CSi(CH$_3$)$_2$.

3.3.2.4 Synthesis of Fmoc-oxazoline-OMe (7)

3.3.2.4.1 Using Burgess reagent

A solution of dipeptide **(6)** (7.16 g, 13.2 mmol) in 131 ml of dry THF (0.1 M) was treated with Burgess reagent (3.46 g, 14.5 mmol) and heated to reflux at 75 ^{0}C for 2 h. The solvent was evaporated to provide an oily residue that was purified using dry column chromatography in EtOAc/hexane system to yield oxazoline **(7)** 25-70% as a white solid (see Appendices 18-22), mp 82-83 ^{0}C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.90 (d, *J* = 7.5

Hz, 2H; 2x Fmoc H Ar), 7.73 (t, *J* = 7.0 Hz, 2H; 2x Fmoc H Ar), 7.68 (d, *J* = 9.2 Hz, 1H; NH Fmoc), 7.42 (t, *J* = 7.4 Hz, 2H; 2x Fmoc H Ar), 7.32 (t, *J* = 7.0 Hz, 2H; 2x Fmoc H Ar), 4.77 (t, *J* = 9.3 Hz, 1H; NHCHCH$_2$O), 4.48 – 4.38 (m, 2H; NCHCH$_2$O), 4.35 (m, 1H; NCHCH$_2$OSi), 4.29 (d, *J* = 6.8 Hz, 2H; Fmoc CH$_2$), 4.22 (t, *J* = 6.7 Hz, 1H; Fmoc CH), 3.83 (dd, *J* = 10.1, 5.6 Hz, 1H; NHCHCH$_2$OSi), 3.74 (dd, *J* = 10.1, 7.4 Hz, 1H; NHCHCH$_2$OSi), 3.68 (s, 3H; OCH$_3$), 0.83 (s, 9H; (CH$_3$)$_3$CSi(CH$_3$)$_2$), 0.02 (s, 6H; (CH$_3$)$_3$CSi(CH$_3$)$_2$). ^{13}C NMR (126 MHz, DMSO) δ 171.46; (CO)OCH$_3$, 167.50; C=N (oxazoline), 156.3; OCONH, [144.26, 141.17]; C Fmoc Ar, 128.09, 127.50, 125.76, 120.56]; CH Fmoc Ar , 69.92; CH$_2$CH (oxazoline), 68.06; CH$_2$CH (oxazoline), 66.31; Fmoc CH$_2$, 63.24; CHCH$_2$OSi, 52.61; OCH$_3$, 51.67; CHCH$_2$OSi, 47.06; Fmoc CH, 26.10; (CH$_3$)$_3$CSi(CH$_3$)$_2$, 18.33; (CH$_3$)$_3$CSi(CH$_3$)$_2$, -5.00; (CH$_3$)$_3$CSi(CH$_3$)$_2$.

3.3.2.4.2 Using DAST reagent

Dipeptide **(6)** (1.5 g, 2.7 mmol) was dissolved in dry DCM (27 ml, 0.1 M), and the mixture was cooled to -78 ^{0}C under N_2 atmosphere. DAST reagent (0.28 ml, 2.97 mmol) was added dropwise to the solution and stirred for 1h. Anhydrous K_2CO_3 (0.55 g, 4.05 mmol) was added to the mixture in one portion and stirred for 1 h at the same temperature then for another h while allowing the reaction to warm to room temperature. The mixture was poured into saturated aqueous $NaHCO_3$, and the aqueous layer was extracted with DCM twice. The organic layer was dried over $MgSO_4$, filtered and concentrated *in vacuo*. The residue was purified by column chromatography using an EtOAc/hexane system to give the desired product **(7)** in a good yield 80%. NMR analysis as mentioned above.

3.3.2.5 Synthesis of H_2N-oxazole-OMe using DBU/$BrCCl_3$ (8)

Oxazoline **(7)** (1.2 g, 2.29 mmol) was dissolved in DCM (30 ml, 0.1M), and the mixture was cooled to 0 ^{0}C under N_2 atmosphere. DBU (1.23 ml, 8.24 mmol) and $BrCCl_3$ (0.82 ml, 8.24 mmol) were added to the mixture dropwise. The reaction stirred at room temperature for 24 h, then the mixture was diluted with DCM. The organic layer was

washed with H_2O, brine, dried over $MgSO_4$, filtered and the filtrate was concentrated *in vacuo*. The residue was purified by trituration with ether and the filtrate was concentrated to provide NH_2-Oxazole-OMe **(8)** as a brown oil in 80% yield (see Appendix 23). ^{1}H NMR (500 MHz, Methanol-d_4) δ 8.72 (s, 1H; C<u>H</u> (oxazole)), 4.84-4.80 (m, 1H; $NH_2C\underline{H}CH_2OSi$), 4.22 (dd, J = 16.9, 4.4 Hz, 1H; $NH_2CHC\underline{H}_2OSi$), 3.92 (s, 3H; $OC\underline{H}_3$), 3.90 (m, 1H; $NH_2CHC\underline{H}_2OSi$), 0.88 (s, 9H; $(\underline{CH}_3)_3CSi(CH_3)_2$), 0.08 (d, J = 14.0 Hz, 6H; $(CH_3)_3CSi(\underline{CH}_3)_2$).

3.3.2.6 One pot synthesis from dipeptide to H_2N-oxazole-OMe using DAST reagent and DBU/ $BrCCl_3$ (8)

DAST reagent (0.35 ml, 3.51 mmol) was injected into a solution of dipeptide **(6)** (1.5 g, 2.7 mmol) in DCM (27 ml, 0.1 M) at -78 ^{0}C under N_2 atmosphere. After stirring for 1 h at the same temperature, TLC observations determined that the reaction was completed. DBU (1.3 ml, 8.8 mmol) was added to the mixture at -40 ^{0}C and stirred for 20 min. Then, $BrCCl_3$ (0.86 ml, 8.8 mmol) was added at 0 ^{0}C and stirred overnight at room temperature. The mixture was then washed with H_2O, dried over $MgSO_4$ and filtered. The filtrate was concentrated *in vacuo* to leave a brown residue that was purified by trituration with hexane to provide the desired product **(8)** in 70%. NMR analysis as mentioned above.

3.3.2.7 Synthesis of Fmoc-oxazole-OMe (9)

3.3.2.7.1 Introducing Fmoc group to the H_2N-oxazole-OMe

H_2N-Oxazole-OMe **(8)** (50 mg, 0.16 mmol) was dissolved in DMF (1.6 ml, 0.1 M), and the reaction mixture was cooled to 0 ^{0}C under a nitrogen atmosphere. Na_2CO_3 (33 mg, 0.32 mmol) in H_2O (1.6 ml) was added to the reaction. Then Fmoc-OSu (84 mg, 0.24 mmol) in DMF (1.6 ml) was added at 0 ^{0}C and the mixture was stirred overnight at room temperature. The workup was completed by extraction with ether twice, and the aqueous

layer was acidified with 0.5 N $NaHSO_4$ to pH=3 at 0 ^{0}C. Following this process, the aqueous layer was extracted with ether. The combined organic layer was washed with brine and dried over $MgSO_4$. The crude was purified via column chromatography using an EtOAc/hexane system to give Fmoc-oxazole-OMe **(9)** as a white solid in 30% yield (see Appendices 24-28), mp 96-97 ^{0}C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.85 (s, 1H; C<u>H</u> (oxazole)), 8.08 (d, *J* = 8.4 Hz, 1H; N<u>H</u> Fmoc), 7.89 (d, *J* = 7.5 Hz, 2H; 2x Fmoc <u>H</u> Ar), 7.71 (t, *J* = 7.2 Hz, 2H; 2x Fmoc <u>H</u> Ar), 7.42 (t, *J* = 7.4 Hz, 2H; 2x Fmoc <u>H</u> Ar), 7.32 (t, *J* = 7.5 Hz, 2H; 2x Fmoc <u>H</u> Ar), 4.88 – 4.79 (m, 1H; NHC<u>H</u>CH_2OSi), 4.32 (d, *J* = 7.0 Hz, 2H; Fmoc C<u>H</u>$_2$), 4.22 (t, *J* = 7.1 Hz, 1H; Fmoc C<u>H</u>), 3.99 (dd, *J* = 10.6, 7.0 Hz, 1H; NHCHC<u>H</u>$_2$OSi), 3.91 (dd, *J* = 9.4, 7.2 Hz, 1H; NHCHC<u>H</u>$_2$OSi), 3.81 (s, 3H; OC<u>H</u>$_3$), 0.79 (s, 9H; (<u>CH</u>$_3$)$_3$CSi$(CH_3)_2$), 0.00 (d, *J* = 8.9 Hz, 6H; $(CH_3)_3$CSi(<u>CH</u>$_3$)$_2$). ^{13}C NMR (126 MHz, DMSO) δ 163.52; (<u>C</u>O)OCH_3, 161.50; <u>C</u>=N (oxazole), 156.31; O<u>C</u>ONH, 146.05; H<u>C</u>=C (oxazole), [144.19, 141.18]; <u>C</u> Fmoc Ar , 132.70; HC=<u>C</u> (oxazole), [128.10, 127.50, 125.68, 120.57]; <u>C</u>H Fmoc Ar , 66.33; Fmoc <u>C</u>H_2, 63.33; CH<u>C</u>H_2OSi, 52.27; O<u>C</u>H_3, 51.54; <u>C</u>HCH_2OSi, 47.05; Fmoc <u>C</u>H, 26.04; (<u>C</u>$H_3)_3$CSi$(CH_3)_2$, 18.26; $(CH_3)_3$<u>C</u>Si$(CH_3)_2$, -5.03; $(CH_3)_3$CSi(<u>C</u>$H_3)_2$.

3.3.2.7.2 Using MnO_2

Oxazoline **(7)** (1.7 g, 3.25 mmol) was dissolved in anhydrous cyclohexane (19 ml). Then, activated MnO_2 (8.5 g, mmol) and molecular sieves (100 Wt%) were added and the reaction mixture was stirred at 80 ^{0}C for 2 days. Following this process, the reaction mixture was filtered through a short Celite column and washed with EtOAc. The solvent was evaporated *in vacuo* and the resulting residue was purified by column chromatography using an EtOAc/hexane system to provide oxazole **(9)** in 20-40% yield as a pale yellow solid. NMR analysis as mentioned above.

3.3.2.7.3 Using CuBr and $Cu(OAc)_2$

CuBr (0.6 g, 4.19 mmol) and $Cu(OAc)_2$ (0.76 g, 4.19 mmol) were added to the oxazoline **(7)** (2 g, 3.81 mmol) in a 50 ml round bottomed flask with three necks. The flask was evacuated and then filled with argon three times. Then, benzene (23.8 ml, 0.1 M) was syringed into the flask and the reaction was stirred and warmed at 60 ^{0}C. *Tert*-butyl perbenzoate (1.09 ml, 5.71 mmol) was added gradually over 15 minutes. Following this, the mixture was heated to reflux at 80 ^{0}C for 8 h. After the reaction mixture was cooled to room temperate, ethyl acetate (50 ml) was added and then washed with 10% NH_4OH solution to remove the copper salts. The aqueous layer was extracted with ethyl acetate (3×50ml), and the organic extracts were collected and washed with H_2O and brine. Then the combined organic extract was dried over $MgSO_4$ and the solvent was removed under reduced pressure. The crude product was subjected to column chromatography on SiO_2 using an EtOAc/hexane system (20:80) to give oxazole **(9)** as a pale yellow solid in 20-43% yield. NMR analysis as mentioned above.

3.3.2.8 Synthesis of Fmoc-oxazole-COOH (10)

A solution of the Fmoc-oxazole-OMe **(9)** (1.8 g, 3.4 mmol) in *i*-PrOH/H_2O (7:3, 0.1 M) was treated with NaOH (0.16 g, 4.0 mmol) and $CaCl_2$ (0.95 g, 8.6 mmol). After stirring at room temperature for 6 h, the reaction mixture was neutralized with 1 N $NaHSO_4$. The compound was extracted with EtOAc and the organic layer was washed first with H_2O and then brine. Once this process was complete, the organic layer was dried over $MgSO_4$, filtered and all of the solvent was evaporated *in vacuo*. The crude peptide acid **(10)** was obtained as a white solid in 70% yield and used in the next step without further purification (see Appendix 29). ^{1}H NMR (500 MHz, Methanol-d_4) δ 8.21 (s, 1H; C<u>H</u> (oxazole)), 7.80 (d, J = 7.4 Hz, 2H; 2x Fmoc <u>H</u> Ar), 7.70 – 7.61 (m, 2H; 2x Fmoc <u>H</u> Ar),

7.42 – 7.37 (t, *J* = 6.8 Hz, 2H; 2x Fmoc H Ar), 7.34 – 7.26 (m, 2H; 2x Fmoc H Ar), 5.01 – 4.96 (m, 1H; NHCHCH$_2$OSi), 4.60-4.58 (m, 1H; Fmoc CH), 4.45 – 4.32 (m, 2H; Fmoc CH$_2$), 4.06 – 3.94 (m, 2H; NHCHCH$_2$OSi), 0.84 (s, 9H; (CH$_3$)$_3$CSi(CH$_3$)$_2$), 0.03 (d, *J* = 5.9 Hz, 6H; (CH$_3$)$_3$CSi(CH$_3$)$_2$).

3.3.2.9 Synthesis of Fmoc-dioxazole-OMe (11)

A solution of Fmoc-oxazole-COOH **(10)** (1.2 g, 2.4 mmol) in DCM (0.1 M, 24 ml) was treated with HBTU (1.07 g, 2.83 mmol) at room temperature. After that, the reaction mixture was cooled to 0 ^{0}C and DIEA (0.90 ml, 5.19 mmol) was added under a nitrogen atmosphere. After 5 minutes of stirring, NH$_2$-oxazole-OMe **(8)** (0.70 g, 2.4 mmol) was added to the mixture and stirred at room temperature overnight. The solvent was evaporated and the resulting residue was dissolved in EtOAc and washed with 0.5 N NaHSO$_4$, NaHCO$_3$, H$_2$O and brine. The organic layer was dried over MgSO$_4$, filtered and concentrated under reduced pressure to obtain the crude. The crude was purified by column chromatography using an EtOAc/hexane system 60:40 to provide the dioxazole **(11)** as a white solid in 35-50% yield (see Appendices 30-33), mp 179-180 ^{0}C. ^{1}H NMR (500 MHz, Methanol-*d*$_4$) δ 8.50 (s, 1H; CH (oxazole)), 8.42 (s, 1H; CH (oxazole)), 7.80 (d, *J* = 7.5 Hz, 2H; 2x Fmoc H Ar), 7.72 – 7.61 (m, 2H; 2x Fmoc H Ar), 7.38 (t, *J* = 7.1 Hz, 2H; 2x Fmoc H Ar), 7.34 – 7.26 (m, 2H; 2x Fmoc H Ar), 5.41 (t, *J* = 5.1 Hz, 1H; NHCHCH$_2$OSi), 4.99 (t, *J* = 5.7 Hz, 1H; NHCHCH$_2$OSi), 4.41 (d, *J* = 6.2 Hz, 2H; Fmoc CH$_2$), 4.23 (t, *J* = 6.8 Hz, 1H; Fmoc CH), 4.15 (dd, *J* = 7.3, 5.1 Hz, 2H; NHCHCH$_2$OSi), 4.10 – 3.96 (m, 2H; NHCHCH$_2$OSi), 3.88 (s, 3H; OCH$_3$), 0.85 (s, 18H; 2x (CH$_3$)$_3$CSi(CH$_3$)$_2$), 0.04 (d, *J* = 12.3 Hz, 12H; 2x (CH$_3$)$_3$CSi(CH$_3$)$_2$). ^{13}C NMR (126 MHz, MeOD) δ 162.99; (CO)OCH$_3$, 162.78; CONH amide, 161.37; C=N (oxazole), 161.00; C=N (oxazole), 156.83; OCONH, 144.89; HC=C (oxazole), 143.79; C Fmoc Ar, 142.14; HC=C (oxazole), 141.20; C Fmoc Ar, 135.39; HC=C (oxazole), 132.87; HC=C (oxazole), [127.39, 126.75, 124.77, 119.55]; CH Fmoc Ar, 66.71; Fmoc CH$_2$, 63.23;

$CH\underline{C}H_2OSi$, 51.46; $\underline{C}HCH_2OSi$, 51.13; $O\underline{C}H_3$, 49.29; Fmoc $\underline{C}H$, 24.81; $(\underline{C}H_3)_3CSi(CH_3)_2$, 17.58; $(CH_3)_3\underline{C}Si(CH_3)_2$, -6.80; $(CH_3)_3CSi(\underline{C}H_3)_2$.

3.3.2.10 Synthesis of Fmoc-tetraoxazole-OMe

3.3.2.10.1 The first route using two dioxazoles

3.3.2.10.1.1 Synthesis of Fmoc-dioxazole-COOH (12)

Once dioxazole **(11)** (0.16 g, 0,205 mmol) was dissolved in *i*-PrOH/H_2O (7:3, 0.1 M), NaOH (0.01 g, 0.247 mmol) and $CaCl_2$ (0.05 g, 0.512 mmol) were added to the mixture and stirred overnight at room temperature. The reaction was neutralized with 1 N $NaHSO_4$ under an ice-bath to pH = 2, followed by extraction with EtOAc. The aqueous layer was re-extracted with EtOAc twice, and then the organic layers were collected and washed with H_2O, brine, dried over $MgSO_4$, and filtered. The filtrate was concentrated *in vacuo* to give a free acid **(12)** as a white solid in 70% yield (mp 195 ^{0}C), and the crude acid was used for the next reaction without further purification (see Appendix 34). ^{1}H NMR (500 MHz, Methanol-d_4) δ 8.42 (s, 2H; 2x C$\underline{H}$ (oxazole)), 7.81 (d, J = 6.4 Hz, 2H; 2x Fmoc $\underline{H}$ Ar), 7.72 – 7.65 (t, J = 6.6 Hz, 2H; 2x Fmoc $\underline{H}$ Ar), 7.41 (t, J = 7.3 Hz, 2H; 2x Fmoc $\underline{H}$ Ar), 7.35 – 7.28 (t, J = 7.2 Hz, 2H; 2x Fmoc $\underline{H}$ Ar), 5.46 – 5.38 (m, 1H; NHC$\underline{H}CH_2OSi$), 5.02 – 4.95 (m, 1H; NHC$\underline{H}CH_2OSi$), 4.43 (d, J = 6.9 Hz, 2H; Fmoc C$\underline{H_2}$), 4.28 – 4.20 (t, J = 6.4 Hz, 1H; Fmoc C$\underline{H}$), 4.20 – 4.11 (m, 3H; NHCHC$\underline{H_2}$OSi), 4.11 – 3.99 (m, 2H; NHCHC$\underline{H_2}$OSi), 0.86 (s, 19H; 2x $(\underline{CH_3})_3CSi(CH_3)_2$), 0.09 – 0.02 (m, 12H; 2x $(CH_3)_3CSi(\underline{CH_3})_2$).

3.3.2.10.1.2 Synthesis of H_2N-dioxazole-OMe (13)

Once dioxazole **(11)** (0.15 g, 0.189 mmol) was dissolved in DCM (0.1 M, 2 ml), the mixture was cooled to 0 ^{0}C and DBU (0.05 ml, 0.378 mmol) was added to the mixture and left to stir overnight at room temperature. The mixture was diluted with DCM and washed with H_2O. The aqueous layer was re-extracted with DCM, and the organic layers were washed with brine, dried over $MgSO_4$, and filtered. The filtrate was concentrated *in vacuo*, and the crude amine **(13)** in 75% yield (mp 200 ^{0}C) that was used for the next step without purification (see Appendix 35). ^{1}H NMR (500 MHz, Methanol-d_4) δ 8.56 (s, 1H; C<u>H</u> (oxazole)), 8.42 (s, 1H; C<u>H</u> (oxazole)), 5.42 (t, J = 3.8 Hz, 1H; NHC<u>H</u>CH_2OSi), 4.18 (dd, J = 12.9, 4.8 Hz, 2H; NHCHC<u>H_2</u>OSi), 4.15 – 4.09 (m, 1H; NHC<u>H</u>CH_2OSi), 4.06 – 3.93 (m, 2H; NHCHC<u>H_2</u>OSi), 3.90 (s, 3H; OC<u>H_3</u>), 0.87 (d, J = 8.5 Hz, 18H; 2x (<u>CH_3</u>)$_3$CSi(CH_3)$_2$), 0.12 – -0.06 (m, 12H; 2x (CH_3)$_3$CSi(<u>CH_3</u>)$_2$).

3.3.2.10.1.3 Synthesis of Fmoc-tetraoxazole-OMe from two dioxazoles (14)

A solution of Fmoc-dioxazole-COOH **(12)** (0.157 g, 0.20 mmol) in DCM (0.1 M, 2 ml) was treated with HBTU (0.1 g, 0.24 mmol) at room temperature. Following this, the reaction mixture was cooled to 0 ^{0}C and DIEA (0.06 ml, 0.44 mmol) was added under a nitrogen atmosphere. After being stirred for 5 minutes, NH_2-dioxazole-OMe **(13)** (0.11 g, 0.20 mmol) was added to the mixture and then stirred at room temperature for 12 h. The reaction mixture was concentrated and the resulting residue was dissolved in EtOAc and washed with 0.5 N $NaHSO_4$, $NaHCO_3$, H_2O and brine. The organic layer was dried over $MgSO_4$, filtered and concentrated under reduced pressure to obtain the crude. The crude was purified by column chromatography using an EtOAc/hexane system 60:40 to get the tetraoxazole **(14)** as a white solid in 80% yield (see Appendices 36-40), mp 180-190 ^{0}C. ^{1}H NMR (500 MHz, Chloroform-d) δ 8.28 – 8.13 (m, 4H; C<u>H</u> (oxazole)), 7.83 – 7.59 (m,

5H; 2x Fmoc H Ar, 3x NH amide), 7.42 (t, *J* = 7.1 Hz, 2H; 2x Fmoc H Ar), 7.33 (t, *J* = 7.1 Hz, 2H; 2x Fmoc H Ar), 5.69 (d, *J* = 8.2 Hz, 1H; NH Fmoc), 5.46 (m, 3H; 3x NHCHCH$_2$OSi), 5.08 (brd, 1H; NHCHCH$_2$OSi), 4.45 (m, 2H; Fmoc CH$_2$), 4.31 – 3.87 (m, 13H; NHCHCH$_2$OSi, Fmoc CH, OCH$_3$), 0.94 – 0.78 (m, 37H; 4x (CH$_3$)$_3$CSi(CH$_3$)$_2$), 0.12 – -0.05 (m, 24H; 4x (CH$_3$)$_3$CSi(CH$_3$)$_2$). ^{13}C NMR (126 MHz, $CDCl_3$) δ 163.11; (CO)OCH$_3$, 162.34; CONH amide, 161.95; C=N (oxazole), 161.84; C=N (oxazole), 161.51; C=N (oxazole), 160.19; C=N (oxazole), 155.74; OCONH, 144.05; HC=C (oxazole), 143.78; C Fmoc Ar, 143.56; C Fmoc Ar, 141.88; HC=C (oxazole), 141.77; HC=C (oxazole), 141.66; HC=C (oxazole), 141.31; HC=C (oxazole), 135.79; HC=C (oxazole), 135.66; HC=C (oxazole), 135.25; HC=C (oxazole), 133.53; HC=C (oxazole), [127.80, 127.08, 125.04, 120.07]; CH Fmoc Ar, 67.36 Fmoc CH$_2$, 64.18; CHCH$_2$OSi, 63.99; CHCH$_2$OSi, 63.89; CHCH$_2$OSi, 63.79; CHCH$_2$OSi, 52.24; OCH$_3$, 51.52; CHCH$_2$OSi, 49.15; CHCH$_2$OSi, 49.05; CHCH$_2$OSi, 48.96; CHCH$_2$OSi, 47.11; Fmoc CH, 25.66; (CH$_3$)$_3$CSi(CH$_3$)$_2$, 18.09; (CH$_3$)$_3$CSi(CH$_3$)$_2$, -5.50; (CH$_3$)$_3$CSi(CH$_3$)$_2$.

3.3.2.10.2 The second route to generate the tetraoxazole

3.3.2.10.2.1 Synthesis of Fmoc-trioxazole-OMe (15)

(0.627 g, 0.78 mmol) of Fmoc-dioxazole-COOH **(12)** and (0.364 g, 0.96 mmol) of HBTU were dissolved in dry DCM (0.1 M, 8 ml) at room temperature. The reaction mixture was then cooled to 0 ^{0}C and (0.3 ml, 1.76 mmol) of DIEA was added dropwise under argon. The mixture was stirred for 5 minutes, followed by the addition of H$_2$N-monooxazole-OMe **(8)** (0.23 g, 0.78 mmol). The reaction mixture was stirred for 1 h at 0 ^{0}C then overnight at room temperature. The mixture was diluted with DCM and washed with 0.5 N $NaHSO_4$, $NaHCO_3$, H_2O and brine. The organic layer was dried over $MgSO_4$, filtered and the solvent was evaporated under reduced pressure to give the crude. The crude was purified by trituration with hot hexane to give the trioxazole **(15)** as a brown fine solid in 75% yield (see Appendices 41-42), mp 245-250 ^{0}C. ^{1}H NMR (500 MHz, Chloroform-*d*) δ 8.31 – 8.09 (brd s, 3H; 3x CH (oxazole)), 7.79 (brd, 3H; 2x Fmoc H Ar, NH amide),

7.70 – 7.59 (brd, 3H; 2x Fmoc H Ar, NH amide), 7.42 (brd, 3H; 2x Fmoc H Ar), 7.33 (brd, 2H; 2x Fmoc H Ar), 5.70 (brd, 1H; NH Fmoc), 5.46 (brd, 1H; NHCHCH$_2$OSi), 5.27 (brd, 1H; NHCHCH$_2$OSi), 5.09 (brd, 1H; NHCHCH$_2$OSi), 4.45 (brd, 2H; Fmoc CH$_2$), 4.34 – 3.64 (m, 10H; 3x NHCHCH$_2$OSi, Fmoc CH, OCH$_3$), 1.02 – 0.63 (s, 27H; 3x (CH$_3$)$_3$CSi(CH$_3$)$_2$), 0.27 – -0.18 (m, 17H; 3x (CH$_3$)$_3$CSi(CH$_3$)$_2$). ^{13}C NMR (126 MHz, $CDCl_3$) δ 162.49; (CO)OCH$_3$, 162.33; CONH amide, 161.41; C=N (oxazole), 160.15; C=N (oxazole), 160.07; C=N (oxazole), 155.18; OCONH, 143.63; C Fmoc Ar, 142.46; HC=C (oxazole), 141.82; HC=C (oxazole), 141.75; HC=C (oxazole), 141.30; C Fmoc Ar, 135.74; HC=C (oxazole), 133.46; HC=C (oxazole), 133.36; HC=C (oxazole), [127.78, 127.07, 125.02, 120.01]; CH Fmoc Ar, 67.40; Fmoc CH$_2$, 64.15; CHCH$_2$OSi, 64.09; CHCH$_2$OSi, 64.00; CHCH$_2$OSi, 51.51; OCH$_3$, 50.81; CHCH$_2$OSi, 49.12; CHCH$_2$OSi, 49.10; CHCH$_2$OSi, 47.12; Fmoc CH, 25.66; (CH$_3$)$_3$CSi(CH$_3$)$_2$, 18.09; (CH$_3$)$_3$CSi(CH$_3$)$_2$, -5.54; (CH$_3$)$_3$CSi(CH$_3$).

3.3.2.10.2.2 Synthesis of Fmoc-trioxazole-COOH (16)

(0.03 g, 0.68 mmol) of NaOH and (0.16 g, 1.4 mmol) of $CaCl_2$ were added to a solution of trioxazole **(15)** in *i*-PrOH/H_2O (7:3, 0.1 M) at room temperature and stirred overnight. Following this, the mixture was acidified with 1 N $NaHSO_4$ and extracted with EtOAc three times. The organic layer was then dried over $MgSO_4$, filtered, and concentrated. The crude acid **(16)** was obtained as a white solid in 72% yield that was used in the next step without further purification (see Appendix 43). ^{1}H NMR (500 MHz, Chloroform-*d*) δ 8.32 – 8.13 (m, 3H; 3x CH (oxazole)), 7.79 (d, *J* = 6.8 Hz, 2H; 2x Fmoc H Ar), 7.65 (m, 3H; 2x Fmoc H Ar, NH amide), 7.42 (t, *J* = 6.6 Hz, 3H; 2x Fmoc H Ar, NH amide), 7.33 (t, *J* = 6.7 Hz, 2H; 2x Fmoc H Ar), 5.70 (brd, 1H, NH Fmoc), 5.49 (brd, 1H; NHCHCH$_2$OSi), 5.27 (brd, 1H; NHCHCH$_2$OSi), 5.09 (brd, 1H; NHCHCH$_2$OSi), 4.46 (d, *J* = 7.6 Hz, 2H; Fmoc CH$_2$), 4.14 (m, 6H; 3x NHCHCH$_2$OSi), 3.93 (t, *J* = 7.6 Hz, 1H;

Fmoc C<u>H</u>), 1.03 – 0.65 (m, 27H; 3x (<u>CH₃</u>)_{<u>3</u>}CSi(CH₃)₂), 0.39 (s, 1H), 0.25 – -0.17 (m, 17H; 3x (CH₃)₃CSi(<u>CH₃</u>)_{<u>2</u>}).

3.3.2.10.2.3 Synthesis of Fmoc-tetraoxazole-OMe from trioxazole and monooxazole (14)

HBTU (0.20 g, 0.53 mmol) was added to a solution of Fmoc-trioxazole-COOH **(16)** (0.44 g, 0.41 mmol) in DCM (0.1 M, 4 ml). The mixture was cooled to 0 ^{0}C under a nitrogen atmosphere, and DIEA (0.1 ml, 0.9 mmol) was added dropwise and stirred for 5 minutes. (0.13 g, 0.41 mmol) amine H_2N-monooxazole-OMe **(8)** was added and the reaction was stirred for 12 h at room temperature. The reaction mixture was diluted with DCM and washed with 0.5 N $NaHSO_4$, $NaHCO_3$, H_2O and brine. The organic layer was dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The crude tetraoxazole was purified via column chromatography on SiO_2 using an EtOAc/hexane system to provide the pure tetraoxazole **(14)** in 70% yield as a white solid. NMR data as mentioned above.

3.3.2.11 Synthesis of Fmoc-tetraoxazole-COOH (17)

Once tetraoxazole **(14)** (0.34 g, 0.25 mmol) was dissolved in *i*-PrOH/H_2O (7:3, 0.1 M), NaOH (0.01 g, 0.3 mmol) and $CaCl_2$ (0.07 g, 0.62 mmol) were added to the mixture and stirred overnight at room temperature. The reaction was neutralized with 1 N $NaHSO_4$ under an ice-bath to pH = 2, followed by extraction with EtOAc. The aqueous layer was re-extracted with EtOAc twice, and then the organic layers were collected and washed with H_2O, brine, dried over $MgSO_4$, and filtered. The filtrate was concentrated *in vacuo* to provide a free acid **(17)** as a white solid in 75% yield, and the crude acid was used for the next reaction without further purification.

3.3.2.12 Synthesis of H_2N-tetraoxazole-COOH (18)

Fmoc-tetraoxazole-COOH **(17)** (0.16 g, 0.205 mmol) was dissolved in DCM (2 ml, 0.1 M) and the mixture was cooled to 0 ^{0}C. DBU (0.112 g, 0.22 mmol) was then added and

the mixture was left to stir overnight at room temperature. The mixture was diluted with DCM and washed with H_2O. Next, the aqueous layer was re-extracted with DCM, and the organic layers were washed with brine, dried over $MgSO_4$, and filtered. The filtrate was concentrated *in vacuo*, and the unprotected tetraoxazole, H_2N-tetraoxazole-COOH **(18)** in 76% yield, was used for the next step without purification (see Appendix 44). ^1H NMR (500 MHz, Chloroform-*d*) δ 11.14 (s, 1H; COOH), 8.30 – 8.13 (brd, 4H; 4x CH (oxazole)), 7.53 (d, *J* = 7.0 Hz, 2H; 2x NH amide), 7.49 (d, *J* = 7.2 Hz, 1H; NH amide), 5.57 – 5.33 (brd, 2H; NHCHCH$_2$OSi), 5.33 – 5.12 (brd, 2H; NHCHCH$_2$OSi), 1.02 – 0.54 (m, 36H; 4x (CH$_3$)$_3$CSi(CH$_3$)$_2$), 0.21 – -0.29 (m, 24H; 4x (CH$_3$)$_3$CSi(CH$_3$)$_2$).

3.3.2.13 Synthesis of the macrocycle tetraoxazole (19)

A solution of the deprotected peptide **(18)** (0.15 g, 0.14 mmol) in dry DMF (70 ml) was added dropwise to a solution of PyOxim (0.08 g, 0.15 mmol) and base DIEA (0.1 ml, 0.44 mmol) in dry DMF (70 ml) at room temperature under a nitrogen atmosphere and stirred for 3 days. The solvent was evaporated under reduced pressure, and then the residue was diluted with DCM. The mixture was washed with 1 N $NaHSO_4$, saturated $NaHCO_3$, water, and brine. The organic layer was dried over $MgSO_4$, filtered, and evaporated *in vacuo*. The crude was purified by column chromatography using an EtOAc/hexane system, to provide the desired compound **(19)** as a yellow oil in 20% yield (see Appendix 45). ^1H NMR (500 MHz, Chloroform-*d*) δ 8.21 (m, 4H; 4x CH (oxazole)), 7.69 (brd, 4H; 4x NH amide), 5.51 – 5.37 (m, 4H; 4x NHCHCH$_2$OSi), 3.40 – 3.29 (m, 4H; 4x NHCHCH$_2$OSi), 3.11 – 3.04 (dd, *J* = 12.9, 3.35 Hz 2H; 2x NHCHCH$_2$OSi), 3.00 (m, 2H; 2x NHCHCH$_2$OSi), 0.99 – 0.71 (m, 36H; 4x (CH$_3$)$_3$CSi(CH$_3$)$_2$), 0.19 – -0.17 (m, 24H; 4x (CH$_3$)$_3$CSi(CH$_3$)$_2$).

3.4 Synthesis of the tetraoxazole macrocyclic peptide with *t*-Bu groups

3.4.1 Synthesis of the dimer Boc-Cys(*t*-Bu)-OH-Ser-OMe (20)

A solution of Boc-L-Cys(*t*-Bu)-OH (5.0 g, 18 mmol) in DCM (180 ml, 0.1 M) was treated with HBTU (8.87 g, 23.3 mmol, 1.2eq) at room temperature. The flask was placed under a nitrogen atmosphere, and the mixture was cooled to 0 ^{0}C. DIEA (10.9 ml, 62.9 mmol) was then added and the mixture was stirred for 5 minutes followed by the addition of H-Ser-L-OMe.HCl (2.80 g, 18 mmol). The resulting mixture was stirred overnight at room temperature and then diluted with DCM, and washed with 0.5 N $NaHSO_4$. The aqueous layer was re-extracted with DCM and the organic layers were collected and washed with $NaHCO_3$, H_2O and brine. The organic layer was then dried over $MgSO_4$, filtered and concentrated *in vacuo*. Finally, the crude was purified by crystallization with hot hexane at 40 ^{0}C overnight to provide the dipeptide **(20)** as a white solid in 100% yield (see Appendices 46-47), mp 145-146 ^{0}C. ^{1}H NMR (500 MHz, Chloroform-*d*) δ 7.20 (d, *J* = 7.3 Hz, 1H; NH amide), 5.42 (brd, 1H; NH Boc), 4.64 (m, 1H; αC<u>H</u>Cys), 4.29 (m, 1H; αC<u>H</u> Ser), 4.07 – 3.98 (dd, *J* = 11, 3.6 Hz 1H; C<u>H$_2$</u> Ser), 3.95 (dd, *J* = 11.5, 3.6 Hz, 1H; C<u>H$_2$</u> Ser), 3.81 (s, 3H; OC<u>H$_3$</u>), 3.03 (dd, *J* = 12.7, 6.4 Hz, 1H; C<u>H$_2$</u> Cys), 2.92 (dd, *J* = 12.8, 5.7 Hz, 1H; C<u>H$_2$</u> Cys), 1.47 (s, 10H; (C<u>H$_3$</u>)$_3$ Boc), 1.35 (s, 9H; (C<u>H$_3$</u>)$_3$ *t*-Bu).^{13}C NMR (126 MHz, $CDCl_3$) δ 170.82; (<u>C</u>O)OCH$_3$, 170.48; <u>C</u>ONH amide, 155.64; C(CH$_3$)$_3$O<u>C</u>O Boc , 80.73; <u>C</u>(CH$_3$)$_3$OCO Boc, 62.59; <u>C</u>H$_2$ Ser, 55.09; α<u>C</u> Cys, 54.54; α<u>C</u> Ser, 52.77; (CO)O<u>C</u>H$_3$, 42.97; <u>C</u>(CH$_3$)$_3$ *t*-Bu, 30.85; C(<u>C</u>H$_3$)$_3$ *t*-Bu, 30.42; <u>C</u>H$_2$ Cys, 28.26; C(<u>C</u>H$_3$)$_3$OCO Boc.

3.4.2 Synthesis of Boc-oxazole-OMe (21)

3.4.2.1 Synthesis of Boc-oxazoline-OMe

(6.8 g, 17.9 mmol) of dipeptide **(20)** was dissolved in dry DCM (0.1 M, 180 ml) and cooled to -78 ^{0}C under N_2. DAST reagent (2.62 ml, 26.81 mmol) was added dropwise over 15 min to the solution and left to stir for 2 h. (4.94 g, 35.74 mmol) of anhydrous K_2CO_3 was then added to the mixture in one portion and stirred for 1 h at the same temperature than for another h allowing the reaction to warm to room temperature. Based on the observations of the TLC, the reaction was complete. The reaction mixture was poured into saturated aqueous $NaHCO_3$, and the aqueous layer was extracted with DCM twice. The combined organic layer was dried over $MgSO_4$, filtered and concentrated *in vacuo*. The residue was used in the next step without further purification.

3.4.2.2 Synthesis of Boc-oxazole-OMe

DBU (5.35 ml, 35.79 mmol) was added to a solution of crude oxazoline in DCM (0.1 M, 180 ml) at 0 ^{0}C under a nitrogen atmosphere. After being stirred for 5 min, $BrCCl_3$ (3.52 ml, 35.75 mmol) was added at the same temperature. The reaction was stirred for 1 h at 0 ^{0}C and then left to stir at room temperature overnight. The reaction mixture was diluted with DCM and washed with NH_4Cl. The aqueous layer was re-extracted with DCM, and the combined organic layer was washed with brine, dried over $MgSO_4$, and filtered. After the solvent was removed under reduce pressure, the residue was purified by column chromatography on SiO_2 with EtOAc/hexane system to give the monooxazole **(21)** as a white solid in 48 % yield in 3 steps (see Appendices 48-49), mp 120-121 ^{0}C.^{1}H NMR (500 MHz, Chloroform-*d*) δ 8.21 (s, 1H; C<u>H</u>= (Oxazole)), 5.49 (brd, 1H; N<u>H</u> Boc), 5.17 (brd, 1H; αC<u>H</u>Cys), 3.93 (s, 3H; OC<u>H$_3$</u>), 3.13 (dd, *J* = 13.3, 5.4 Hz, 1H; C<u>H$_2$</u> Cys), 3.07 (dd, *J* = 13.1, 6.2 Hz, 1H; C<u>H$_2$</u> Cys), 1.45 (s, 9H; (C<u>H$_3$</u>)$_3$ Boc), 1.31 (s, 9H; (C<u>H$_3$</u>)$_3$ *t*-Bu).^{13}C NMR (126 MHz, $CDCl_3$) δ 164.08; (<u>C</u>O)OCH$_3$, 161.43; <u>C</u>=N(Oxazole), 154.69; C(CH$_3$)$_3$O<u>C</u>O Boc, 144.09; <u>C</u>H=C (Oxazole), 133.37; CH=<u>C</u> (Oxazole), 80.35; <u>C</u>(CH$_3$)$_3$OCO Boc, 52.22; COO<u>C</u>H$_3$, 49.17; α<u>C</u> Cys, 42.85; <u>C</u>(CH$_3$)$_3$ *t*-Bu, 32.55; <u>C</u>H$_2$ Cys , 30.88; C(<u>C</u>H$_3$)$_3$ *t*-Bu, 28.27; C(<u>C</u>H$_3$)$_3$OCO Boc.

3.4.3 Synthesis of Boc-oxazole-COOH using LiOH (22)

The protected monooxazole **(21)** (1.5 g, 4.19 mmol) was dissolved in THF (0.1 M, 42 ml) and the solution was cooled to 0 ^{0}C under a nitrogen atmosphere. LiOH.H_2O (0.351 g, 8.38 mmol) in H_2O (0.1 M, 42 ml) was added dropwise over 15 min at 0 ^{0}C. After the reaction being stirred for 3 h, THF was evaporated and the residue was diluted with water. The reaction mixture was acidified with 1 N HCl at 0 ^{0}C to the PH=3 and extracted with EtOAc twice. The organic layers were combined and washed with brine, dried over $MgSO_4$ and filtered. The organic solvent was removed under reduced pressure to give the free acid **(22)** as a yellow solid in 45% yield (see Appendices 50-51), mp 137-139 ^{0}C. ^{1}H NMR (500 MHz, Chloroform-*d*) δ 8.30 (s, 1H; C<u>H</u>= (Oxazole)), 5.73 (brd, 1H; N<u>H</u> Boc), 5.21 (brd, 1H; αC<u>H</u>Cys), 3.13 (dd, *J* = 12.5, 5.0 Hz, 1H; C<u>H$_2$</u> Cys), 3.09 (dd, *J* = 12.8, 6.4 Hz, 1H; C<u>H$_2$</u> Cys), 1.46 (s, 9H; (C<u>H$_3$</u>)$_3$ Boc), 1.32 (s, 9H; (C<u>H$_3$</u>)$_3$ *t*-Bu). ^{13}C NMR (126 MHz, $CDCl_3$) δ 164.65; (<u>C</u>O)OH, 163.97; <u>C</u>=N(Oxazole), 154.96; C$(CH_3)_3$O<u>C</u>O Boc , 145.00; <u>C</u>H=C (Oxazole), 132.84; CH=<u>C</u> (Oxazole), 80.44; <u>C</u>$(CH_3)_3$OCO Boc, 49.21; α<u>C</u> Cys, 42.92; <u>C</u>$(CH_3)_3$ *t*-Bu, 32.43; <u>C</u>H$_2$ Cys, 30.88; C(<u>C</u>H$_3$)$_3$ *t*-Bu, 28.28; C(<u>C</u>H$_3$)$_3$OCO Boc.

3.4.4 Synthesis of HCl.H_2N-oxazole-OMe (23)

(1.5 g, 4.19 mmol) of the monooxazole **(21)** was dissolved in dry THF (0.3 M, 13 ml) and the mixture was cooled to 0 ^{0}C under a nitrogen atmosphere. (34.5 ml, 138 mmol) of 4 M HCl/1,4 dioxane was added to the mixture and stirred for 2 h at room temperature. The solvent was evaporated *in vacuo*, and the oily residue was washed with dry ether. A simple decantation was used to give the free amine **(23)** as a salt in a quantitative yield (see Appendices 52-53), mp 97-99 ^{0}C. ^{1}H NMR (500 MHz, Methanol-d_4) δ 8.70 (s, 1H; C<u>H</u>= (Oxazole)), 4.82 (m, 1H; αC<u>H</u>Cys), 3.92 (s, 3H; OC<u>H$_3$</u>), 3.27 (m, 1H; C<u>H$_2$</u> Cys), 3.22 (dd, *J* = 13.4, 6.9 Hz, 1H; C<u>H$_2$</u> Cys), 1.36 (s, 9H; (C<u>H$_3$</u>)$_3$ *t*-Bu). ^{13}C NMR (126 MHz, MeOD) δ 161.22; (<u>C</u>O)OCH_3, 159.70; <u>C</u>=N (Oxazole), 145.97; <u>C</u>H=C (Oxazole),

133.28; CH=C (Oxazole), 51.37; COOCH$_3$, 48.79; αC Cys, 43.11; C(CH$_3$)$_3$ *t*-Bu, 29.65; C(CH$_3$)$_3$ *t*-Bu, 29.32; CH$_2$ Cys.

3.4.5 Synthesis of Boc-dioxazole-OMe (24)

A solution of free acid Boc-oxazole-COOH **(22)** (0.47 g, 1.4 mmol) in DCM (0.1 M, 14.3 ml) was treated with HBTU (0.65 g, 1.7 mmol) at room temperature. After that, the reaction mixture was cooled to 0 ^{0}C and DIEA (0.8 ml, 4.5 mmol) was added under a nitrogen atmosphere. After 5 minutes of stirring, HCl.H$_2$N-Oxazole-OMe **(23)** (0.4 g, 1.4 mmol) was added to the mixture and stirred at room temperature overnight. The mixture was diluted with DCM and washed once each with 1 N $NaHSO_4$, saturated $NaHCO_3$, H_2O, and brine. The organic layer was dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The resulting crude was purified by column chromatography using an EtOAc/hexane system to afford the desired compound **(24)** as a yellow oil in 80% yield (see Appendices 54-55). ^{1}H NMR (500 MHz, Chloroform-*d*) δ 8.22 (s, 1H; CH= (Oxazole)), 8.16 (s, 1H; CH= (Oxazole)), 7.63 (d, *J* = 9.2 Hz, 1H; NH amide), 5.61 (m, 1H; NH Boc), 5.39 (brd, 1H; αCHCys), 5.14 (brd, 1H; αCHCys), 3.94 (s, 3H; OCH$_3$), 3.25 (dd, *J* = 13.4, 6.05 Hz, 1H; CH$_2$ Cys), 3.18 (dd, *J* = 13.1, 6.85 Hz, 1H; CH$_2$ Cys), 3.15 – 2.98 (m, 2H; CH$_2$ Cys), 1.48 (s, 9H; (CH$_3$)$_3$ Boc), 1.35 (m, 21H; 2x(CH$_3$)$_3$ *t*-Bu). ^{13}C NMR (126 MHz, $CDCl_3$) δ 163.46; (CO)OCH$_3$, 163.01; CONH amide, 161.39; C=N(Oxazole), 159.86; C=N(Oxazole), 154.83; C(CH$_3$)$_3$OCO Boc, 144.17; CH=C (Oxazole), 141.81; CH=C (Oxazole) , 135.46; CH=C (Oxazole), 133.56; CH=C (Oxazole), 133.56; CH=C (Oxazole), 80.49; C(CH$_3$)$_3$OCO Boc, 52.26; COOCH$_3$, 49.09; αC Cys, 47.48; αC Cys, 43.17; C(CH$_3$)$_3$ *t*-Bu, 42.90; CH$_2$ Cys, 32.22; CH$_2$ Cys, 30.95; C(CH$_3$)$_3$ *t*-Bu, 28.31; C(CH$_3$)$_3$OCO Boc.

3.4.6 Synthesis of Boc-dioxazole-COOH (25)

The dioxazole **(24)** (0.616 g, 1.1 mmol) was dissolved in THF (0.1 M, 11 ml), and the mixture was cooled to 0 ^{0}C under N_2 atmosphere. (0.1 g, 2.2 mmol) of LiOH.H_2O in H_2O (0.1 M, 11 ml) was added dropwise to the mixture, and the mixture was stirred for 4 h at room temperature. THF was evaporated under reduced pressure and water was added to the mixture. The mixture was acidified with 1N HCl to pH=3, and then extracted with EtOAc three times. The combined organic layer was washed with brine, dried over $MgSO_4$, and evaporated to dryness to give the acid **(25)** as a white solid in 75% yield (see Appendices 56-57), mp 140-142 ^{0}C. ^{1}H NMR (500 MHz, Chloroform-*d*) δ 8.29 (s, 1H; CH= (Oxazole)), 8.20 (s, 1H; CH= (Oxazole)), 7.69 (d, *J* = 7.4 Hz, 1H; NH amide), 5.62 (d, *J* = 7.0 Hz, 1H; NH Boc), 5.44 (brd, 1H; αCHCys), 5.16 (brd, 1H; αCHCys), 3.26 (dd, *J* = 13.65, 5.45 Hz, 2H; CH_2 Cys), 3.22-3.15 (dd, *J* = 13.65, 6.7 Hz 2H; CH_2 Cys), 1.48 (s, 9H; $(CH_3)_3$ Boc), 1.39 – 1.24 (m, 18H; 2x$(CH_3)_3$ *t*-Bu). ^{13}C NMR (126 MHz, $CDCl_3$) δ 163.52; (CO)OH, 163.10; (CO)NH, 159.98; C=N(Oxazole), 154.89; $C(CH_3)_3OCO$ Boc, 145.02; CH=C (Oxazole), 145.02; CH=C (Oxazole) 135.37; CH=C (Oxazole), 133.02; CH=C (Oxazole), 80.56; $C(CH_3)_3OCO$ Boc, 49.08; αC Cys, 47.50; αC Cys, 43.20; $C(CH_3)_3$ *t*-Bu, 42.92; CH_2 Cys, 32.22; CH_2 Cys, 30.89; $C(CH_3)_3$ *t*-Bu, 28.31; $C(CH_3)_3OCO$ Boc.

3.4.7 Synthesis of Boc-trioxazole-OMe (26)

(0.440 g, 0.77 mmol) of Boc-dioxazole-COOH **(25)** was dissolved in DCM (0.1 M, 8 ml) and the mixture was cooled to 0 ^{0}C under argon. Amine, HCl.H_2N-oxazole-OMe **(23)**, (0.22 g, 0.77 mmol), EDC.HCl (0.18 g, 0.96 mmol), and HOBt (0.13 g, 0.96 mmol) were added successively to the reaction mixture. The mixture was stirred for 5 minutes, followed by the addition of DIEA (0.33 ml, 1.9 mmol). The reaction mixture was stirred for 1 h at 0 ^{0}C then overnight at room temperature. The mixture was diluted with DCM and washed with 0.5 N $NaHSO_4$, $NaHCO_3$, H_2O and brine. The organic layer was dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The crude was purified by

crystallization using hot hexane to give trioxazole **(26)** as a brown solid in 85 % yield (see Appendices 58-59), mp 108-111 ^{0}C. ^{1}H NMR (500 MHz, Chloroform-*d*) δ 8.23 (s, 1H; C<u>H</u>= (Oxazole)), 8.20 (m, 2H; 2xC<u>H</u>= (Oxazole)), 7.65 (d, *J* = 8.7 Hz, 1H; N<u>H</u> amide), 7.55 (d, *J* = 8.5 Hz, 1H; N<u>H</u> amide), 5.65-5.53 (m, 2H; N<u>H</u> Boc, αC<u>H</u>Cys), 5.41 (brd, 1H; αC<u>H</u>Cys), 5.15 (brd, 1H; αC<u>H</u>Cys), 3.93 (s, 3H; OC<u>H$_3$</u>), 3.28 – 3.03 (m, 6H; 3x C<u>H$_2$</u> Cys), 1.47 (s, 9H; (C<u>H$_3$</u>)$_3$ Boc), 1.38 – 1.30 (m, 27H; 3x(C<u>H$_3$</u>)$_3$ *t*-Bu). ^{13}C NMR (126 MHz, $CDCl_3$) δ 163.44; (<u>C</u>O)OCH$_3$, 163.01; (<u>C</u>O)NH, 162.23; <u>C</u>=N(Oxazole), 161.38; <u>C</u>=N(Oxazole), 159.87; <u>C</u>=N(Oxazole), 154.84; C(CH$_3$)$_3$O<u>C</u>O Boc, 144.18; <u>C</u>H=C (Oxazole), 141.99; <u>C</u>H=C (Oxazole), 141.87; <u>C</u>H=C (Oxazole), 135.65; CH=<u>C</u> (Oxazole), 135.47; CH=<u>C</u> (Oxazole), 133.53; CH=<u>C</u> (Oxazole), 80.47; <u>C</u>(CH$_3$)$_3$OCO Boc, 52.24; COO<u>C</u>H$_3$, 49.11; α<u>C</u> Cys, 47.51; α<u>C</u> Cys, 47.39; α<u>C</u> Cys, 43.16; <u>C</u>(CH$_3$)$_3$ *t*-Bu, 42.89; <u>C</u>H$_2$ Cys, 32.16; <u>C</u>H$_2$ Cys, 31.78; <u>C</u>H$_2$ Cys, 30.95; C(<u>C</u>H$_3$)$_3$ *t*-Bu, 28.30; C(<u>C</u>H$_3$)$_3$OCO Boc.

3.4.8 Synthesis of Boc-trioxazole-COOH (27)

(0.05 g, 1.3 mmol) of LiOH.H$_2$O in H$_2$O (0.1 M, 6.5 ml) was added dropwise to a solution of trioxazole **(26)** (0.6 g, 0.65 mmol) in THF (0.1 M, 6.5 ml) at 0 ^{0}C under nitrogen. The mixture was stirred for 1 h at 0 ^{0}C then allowed to warm up to room temperature. Upon completion, THF was evaporated and water was added to the mixture. The mixture was acidified with 1 N $NaHSO_4$ to pH= 2 at 0 ^{0}C and extracted with EtOAc three times. The organic layers were combined and dried over $MgSO_4$, filtered, and concentrated in a *vacuo* to give the free acid **(27)** as a yellow solid in 91% yield (see Appendix 60), mp 156-157 ^{0}C. ^{1}H NMR (500 MHz, Chloroform-*d*) δ 8.32-8.06 (m, 1H; C<u>H</u>= (Oxazole), 7.78-7.48 (brd, 1H; N<u>H</u> amide), 5.52 (m, 3H; 2x αC<u>H</u>Cys, N<u>H</u> Boc), 5.12 (brd, 1H; αC<u>H</u>Cys), 3.09 (m, 6H; 3x C<u>H$_2$</u> Cys), 1.47 (s, 10H; (C<u>H$_3$</u>)$_3$ Boc), 1.34 (m, 28H; 3x(C<u>H$_3$</u>)$_3$ *t*-Bu).

3.4.9 Synthesis of Boc-tetraoxazole-OMe (28)

To a cold solution of Boc-trioxazole-COOH **(27)** (0.5 g, 0.6 mmol) in DCM (0.1 M, 6 ml) was added $HCl.H_2N$-oxazole-OMe, (0.17 g, 0.6 mmol), EDC.HCl (0.14 g, 0.75 mmol), and HOBt (0.1 g, 0.75 mmol). The mixture was stirred for 5 min, then DIEA (0.3 ml, 1.5 mmol) was added slowly. The reaction mixture was stirred for 1 h at 0 ^{0}C then overnight at room temperature. The reaction mixture was diluted with DCM and washed with 0.5 N $NaHSO_4$, $NaHCO_3$, H_2O and brine. The organic layer was dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The crude was purified by column chromatography on SiO_2 using an EtOAc/hexane system to give the tetraoxazole **(28)** as a white solid in 30% yield (see Appendices 61-62). ^{1}H NMR (500 MHz, Chloroform-*d*) δ 8.23 – 8.16 (m, 4H; 4xC<u>H</u>= (Oxazole)), 7.74 (m, 1H; N<u>H</u> amide), 7.62 (d, *J* = 8.1 Hz, 1H; N<u>H</u> amide), 7.57 (d, *J* = 8.2 Hz, 1H; N<u>H</u> amide), 5.62 – 5.52 (m, 3H; 3x <u>α</u>C Cys), 5.43 (brd, 1H; <u>α</u>C Cys), 5.18 – 5.08 (d, *J* = 7.1 Hz, 1H; N<u>H</u> Boc), 3.91 (s, 3H; OC<u>H</u>$_3$), 3.26 – 3.05 (m, 8H; 4x C<u>H</u>$_2$ Cys), 1.44 (s, 12H; (C<u>H</u>$_3$)$_3$ Boc), 1.36 – 1.28 (m, 39H; 4x(C<u>H</u>$_3$)$_3$*t*-Bu). ^{13}C NMR (126 MHz, $CDCl_3$) δ 162.95; (<u>C</u>O)OCH$_3$, 162.55; (<u>C</u>O)NH, 161.30; <u>C</u>=N(Oxazole), 160.48; <u>C</u>=N(Oxazole), 160.37; <u>C</u>=N(Oxazole), 160.12; <u>C</u>=N(Oxazole), 155.10; C(CH$_3$)$_3$O<u>C</u>O Boc, 144.50; <u>C</u>H=C (Oxazole), 142.56; <u>C</u>H=C (Oxazole), 142.49; <u>C</u>H=C (Oxazole), 142.42; <u>C</u>H=C (Oxazole), 135.50; CH=<u>C</u> (Oxazole), 133.43; CH=<u>C</u> (Oxazole), 80.56; <u>C</u>(CH$_3$)$_3$OCO Boc, 52.21; COO<u>C</u>H$_3$, 48.79; <u>α</u>C Cys, 47.65; <u>α</u>C Cys, 47.24; <u>α</u>C Cys, 46.98; <u>α</u>C Cys, 45.89; <u>C</u>H$_2$ Cys, 44.36; <u>C</u>H$_2$ Cys, 44.26; <u>C</u>H$_2$ Cys, 42.91; <u>C</u>(CH$_3$)$_3$, *t*-Bu, 30.90; C(<u>C</u>H$_3$)$_3$ *t*-Bu, 29.66; <u>C</u>H$_2$ Cys, 28.24; C(<u>C</u>H$_3$)$_3$OCO Boc.

3.4.10 Synthesis of Boc-tetraoxazole-COOH (29)

Once Boc-tetraoxazole-OMe **(28)** (0.08 g, 0.085 mmol) was dissolved in THF (0.1 M, 1 ml), $LiOH.H_2O$ (0.01 g, 0.17 mmol) in H_2O (0.1 M, 1 ml) was added to the mixture at 0

^{0}C under a nitrogen atmosphere. The mixture was stirred for 6 h at room temperature. THF was evaporated and the mixture was acidified with 1 N HCl at 0 ^{0}C. The aqueous layer was extracted with EtOAc twice. The combined organic layer was washed with brine, dried over $MgSO_4$, filtered and concentrated *in vacuo*. The free acid, Boc-tetraoxazole-COOH **(29)** was obtained in 90% yield and used in the next step without further purification.

3.4.10 Synthesis of HCl.H_2N-tetraoxazole-COOH (30)

The free acid, Boc-tetraoxazole-COOH **(29)** (0.08 g, 0.085 mmol) was dissolved in dry THF (0.3 M, 0.3 ml) and the mixture was cooled to 0 ^{0}C under a nitrogen atmosphere. (0.7 ml, 2.80 mmol) of 4 M HCl/1,4 dioxane was added and the reaction mixture was stirred for 8 h at room temperature. The solvent was evaporated *in vacuo*. Then, the residue was washed with dry ether, and collected by filtration to provide the free amine **(30)** as a salt in 81% yield (see Appendix 63). ^{1}H NMR (500 MHz, Methanol-d_4) δ 8.57 (s, 4H; 4xC<u>H</u>= (Oxazole)), 5.11 (brd, 3H; 3x <u>αC</u> Cys), 4.20 (m, 1H; <u>αC</u> Cys), 2.42 (m, 9H; 2xC<u>H$_2$</u> Cys).

Chapter 4- Conclusion and outlook

The main emphasis of this study has been to establish a methodology to synthesize new tetraoxazole macrocyclic peptides through solution phase peptide synthesis. The synthesis of the tetraoxazole macrocyclic peptide with TBS groups was demonstrated. After the formation of the dipeptide, the oxazole ring was obtained by cyclodehydration followed by oxidation. Different synthetic routes for the synthesis of the linear tetraoxazole were developed; the convergent approach and the stepwise approach. The final macrocyclization step of the unprotected linear tetraoxazole precursor was accomplished by utilizing the coupling reagent PyOxim under very dilute conditions. Although the target macrocycle was obtained, it was difficult to obtain a pure compound even after purification by column chromatography on SiO_2.

Because of the low yield of the fully protected monooxazole, changing the starting materials as well as the protecting group to Boc and *t*-Bu was done in order to optimize the yield, thus allowing for the synthesis of the tetraoxazole macrocyclic peptide with *t*-Bu groups. In the synthesis of the tetraoxazole macrocyclic peptide with *t*-Bu groups, the yield of the fully protected monooxazole is much higher than that for the synthesis of tetraoxazole macrocyclic peptide with TBS. In addition, the formation of the di, tri-, as well as the tetraoxazoles was preformed easily. After the formation of fully protected tetraoxazole, the methyl ester group was removed successfully. However, when the Boc group was removed using 4 M HCl in dioxane, the *t*-Bu group was also cleaved. As a result, the macrocyclization of the linear tetraoxazole could not be performed.

It would be interesting to scale-up the reaction for the synthesis of the tetraoxazole macrocyclic peptides, and complete the synthesis of tetraoxazole macrocyclic peptide with *t*-Bu groups. The Boc group needs to be removed carefully to avoid the cleavage of the *t*-Bu group. Alternatively, in case of *t*-Bu cleavage, the thiol group could be reprotected prior to cyclization.

The tetraoxazole macrocyclic peptide with TBS groups, unfortunately, was not tested for metal complexation due to impurity. In addition, there were a lot of challenges with the

chemical synthesis of the target compound. However, in using the Boc and *t*-Bu protecting groups, the yields of the intermediate compounds needed to form the tetraoxazole macrocyclic peptide were improved. Once the macrocycle is obtained, perhaps high-performance liquid chromatography could be used to purify the compound, the metal complexation could be evaluated.

With structural similarities to the macrocyclic peptides derived from the marine sources, the tetraoxazole macrocyclic peptide with TBS groups might be utilized as a chelating agent to remove metals. Furthermore, the side chain in the tetraoxazole macrocyclic peptide can be changed to a variety of functional groups; thus, different derivatives can be generated from it without altering the ring structure by the modification of the side chain. In theory, after deprotecting the TBS group using acid or fluoride, different groups can be introduced to the tetraoxazole macrocyclic peptide. Therefore, the synthesis of different derivatives of tetraoxazole macrocyclic peptides can be performed.

In addition, the side chain in the tetraoxazole macrocyclic peptide, which is OTBS, can allow the formation of an octaoxazole macrocycle. After the removal of the protecting group, the side chains of the amino acid can be used for four cyclodehydration reactions in a single step, followed by oxidation reaction. Thus, providing the octa-oxazole macrocycle which potentially could serve an analog to telomestatin. With the structural similarity to telomestatin, the compound could be used as an anticancer therapeutic agent that targets the G-quadruplex. Studies could be performed to test these hypotheses, along with the investigation of metal complexation properties.

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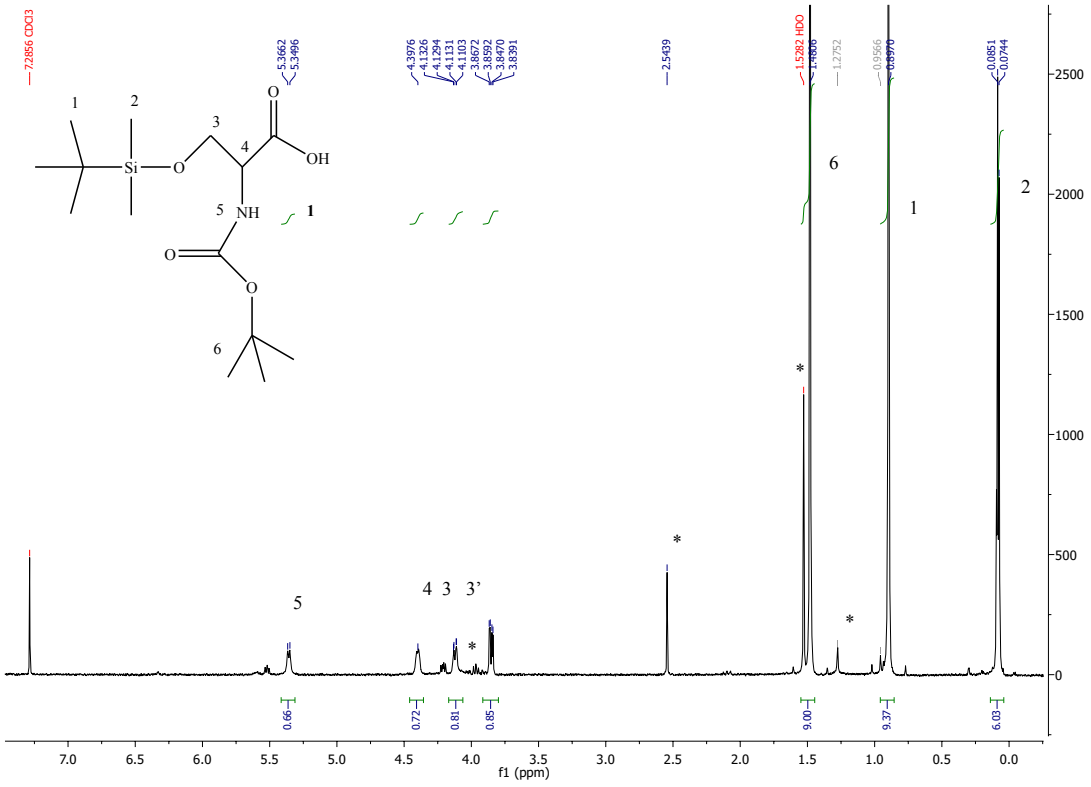
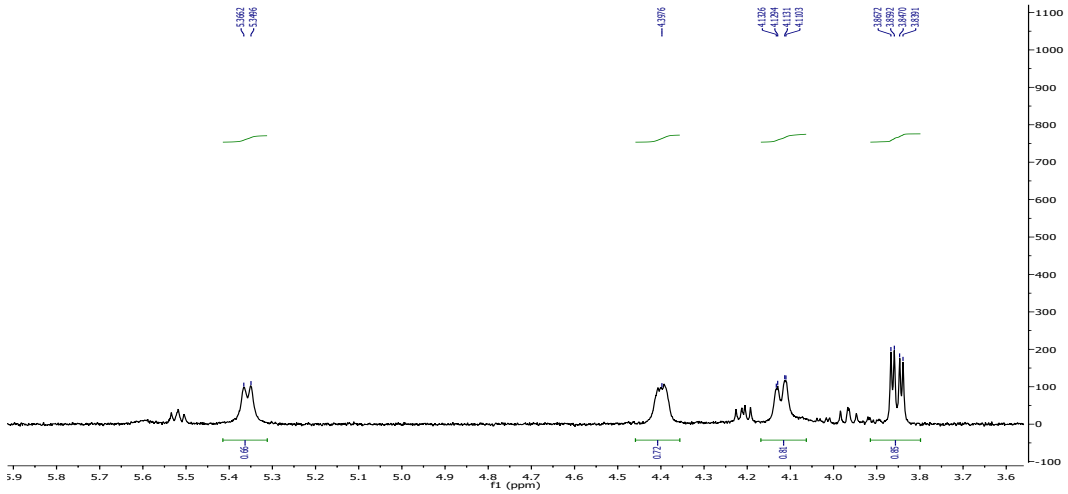
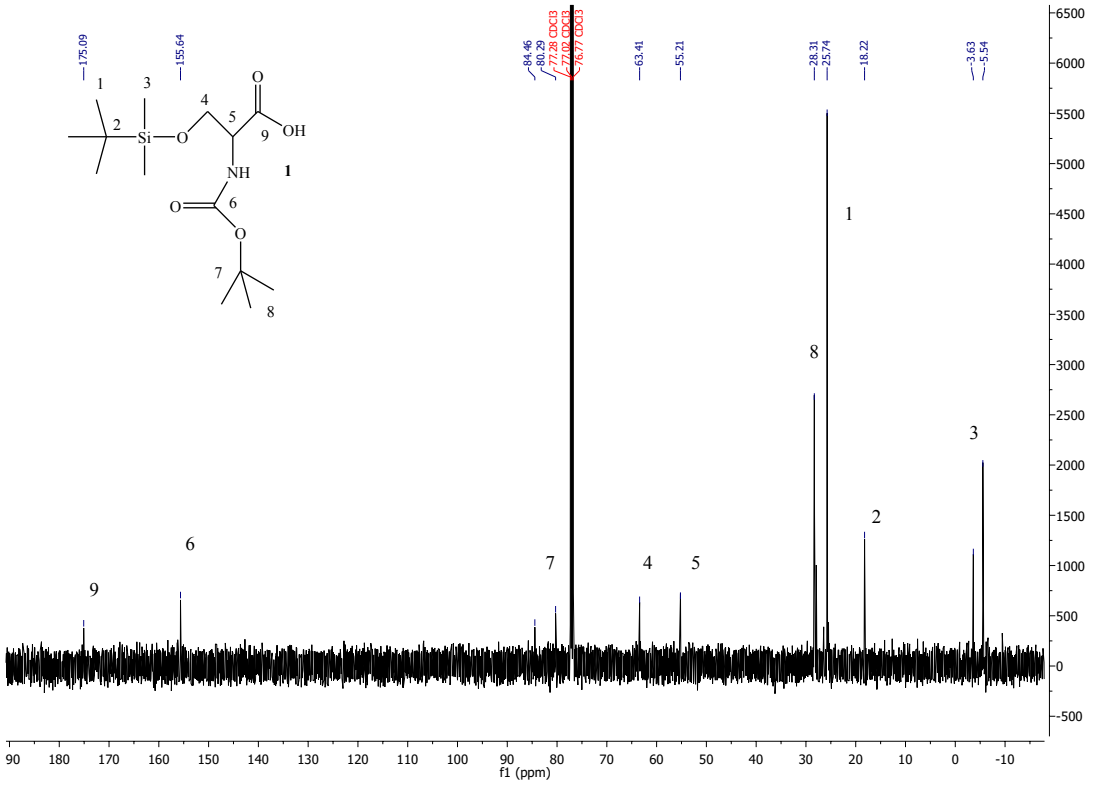
Appendix-Supporting Spectra for Chapter 2



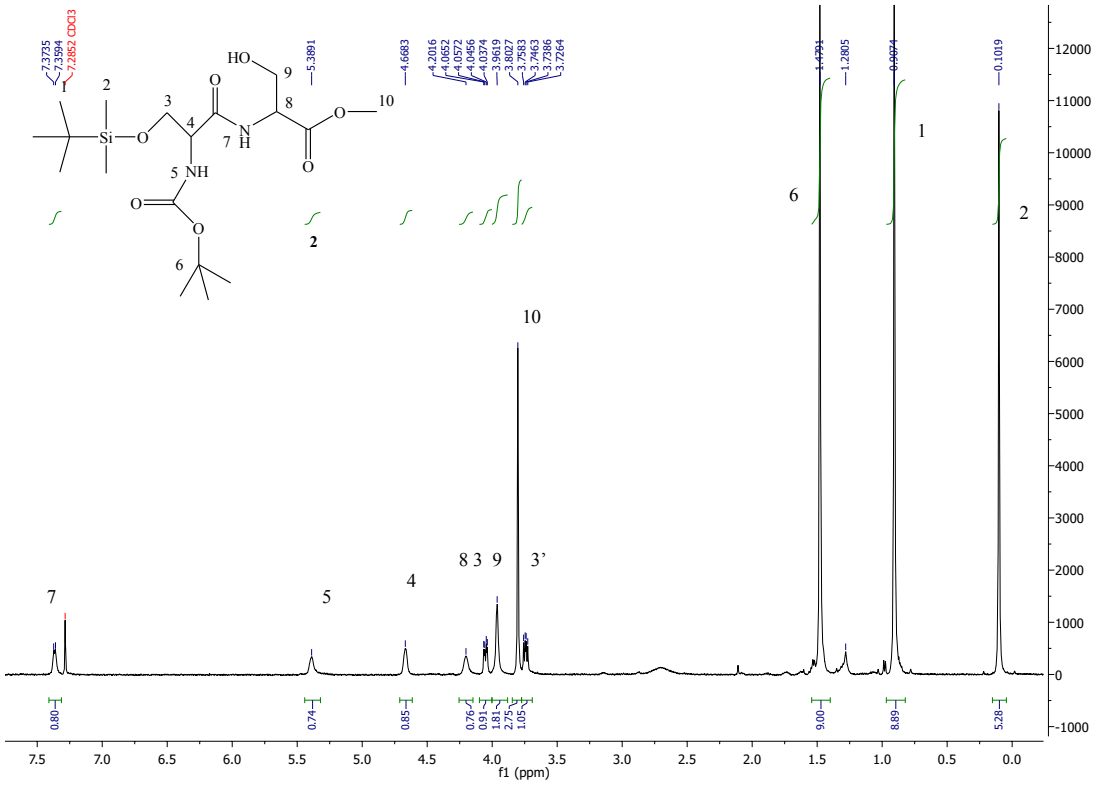
Appendix 1: ^{1}H NMR Boc-Ser-OH(O-TBS) (1).

* Peaks at at 1.24, 2.01 and 4.10 ppm due to EtOAc solvent. * Peak at1.5 ppm due to water.

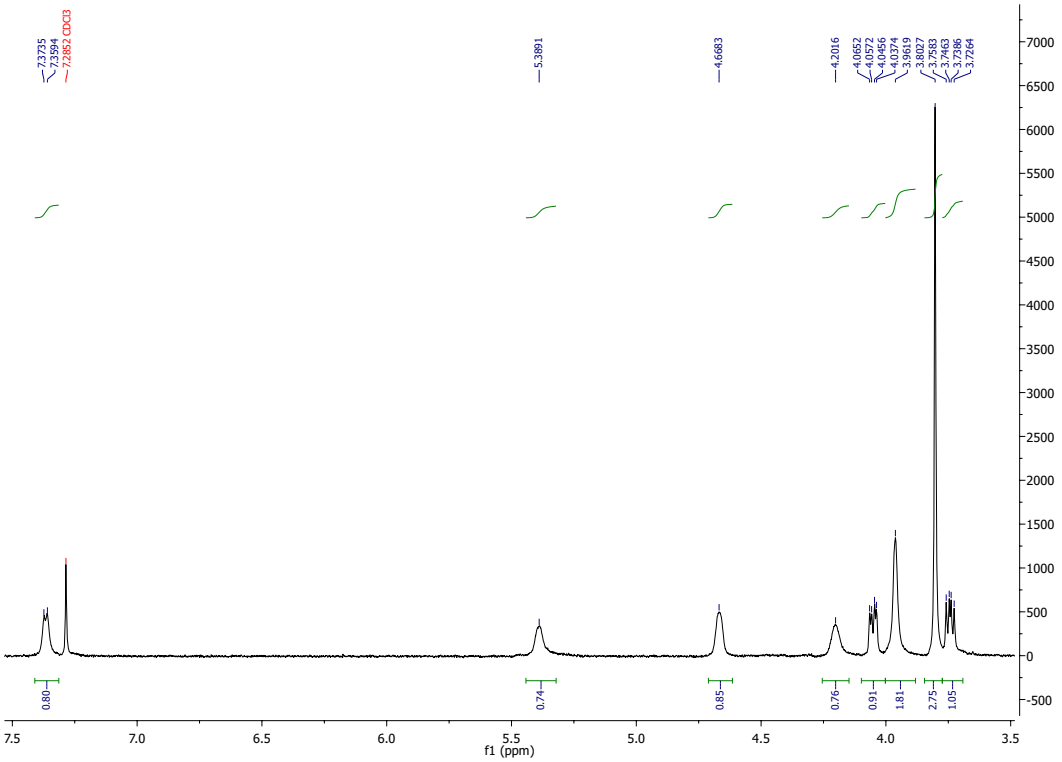


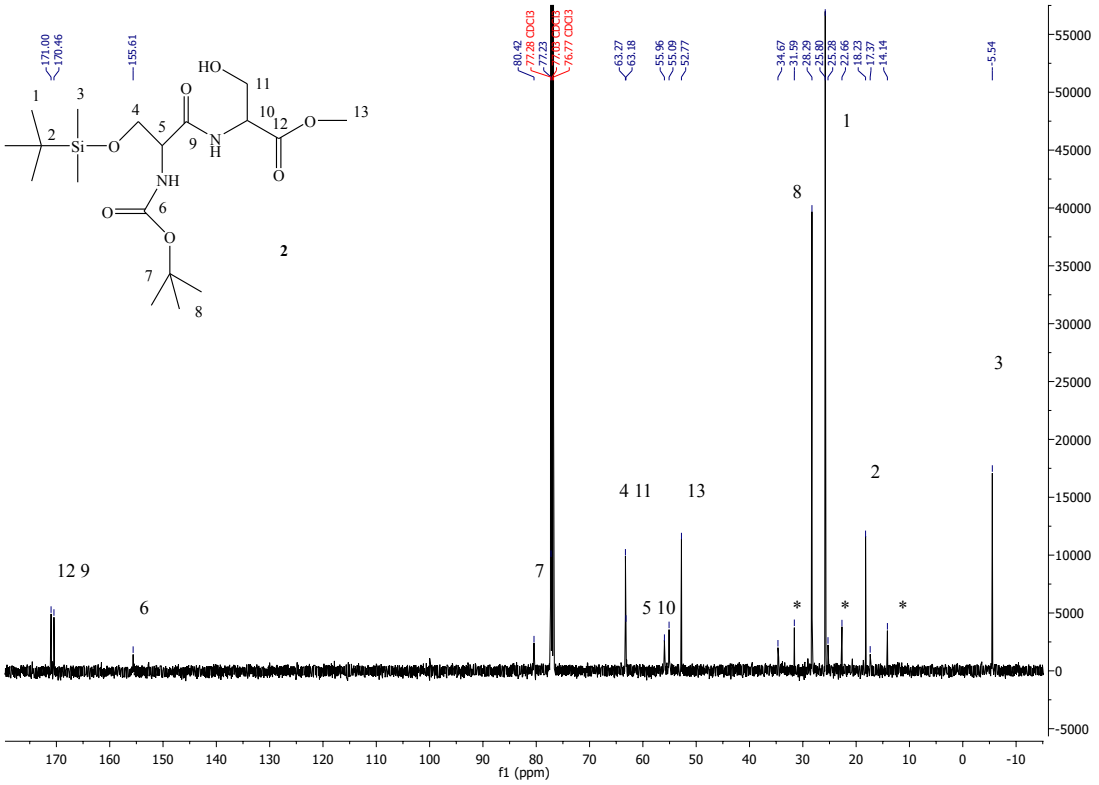


Appendix 2: ^{13}C NMR Boc-Ser-OH(O-TBS) (1).



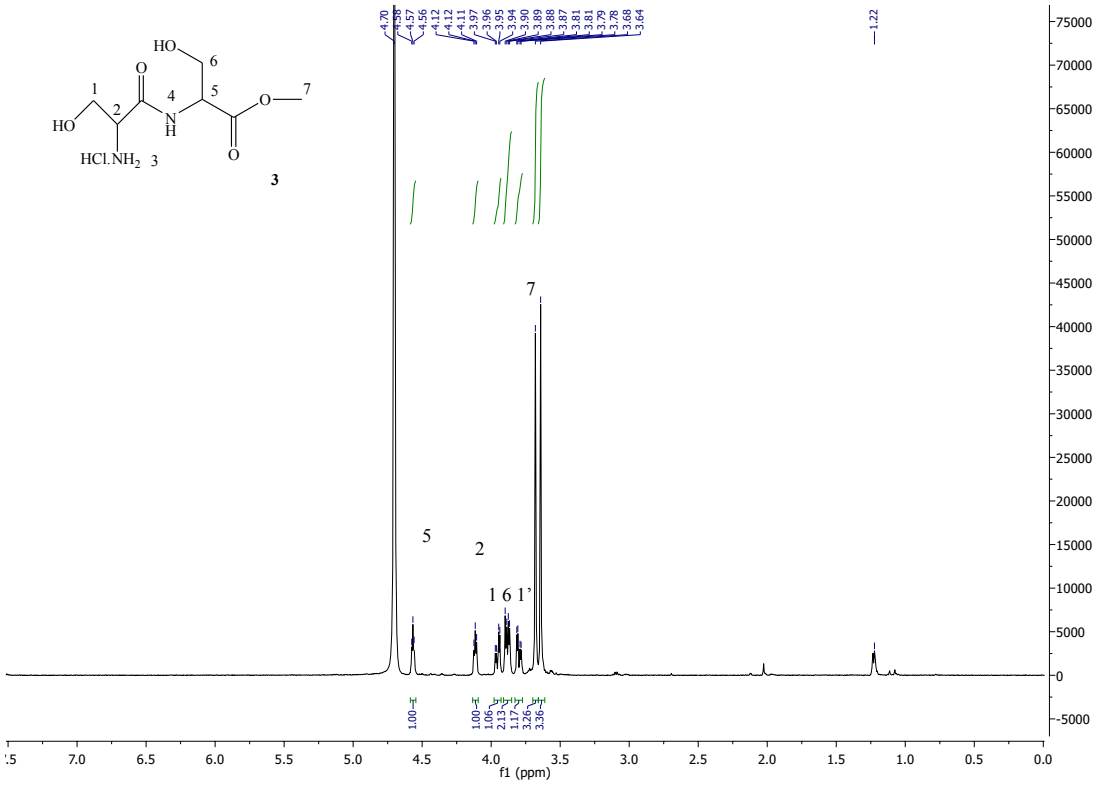
Appendix 3: ^{1}H NMR N-Boc-Ser(O-TBS)-Ser-OMe (2).



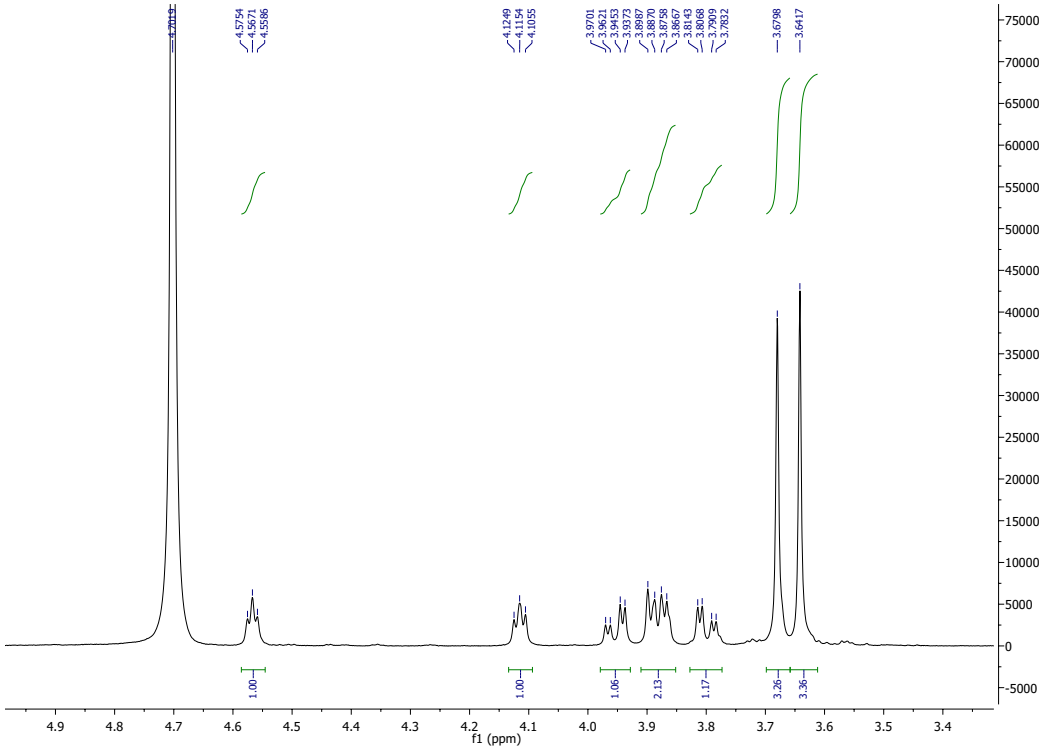


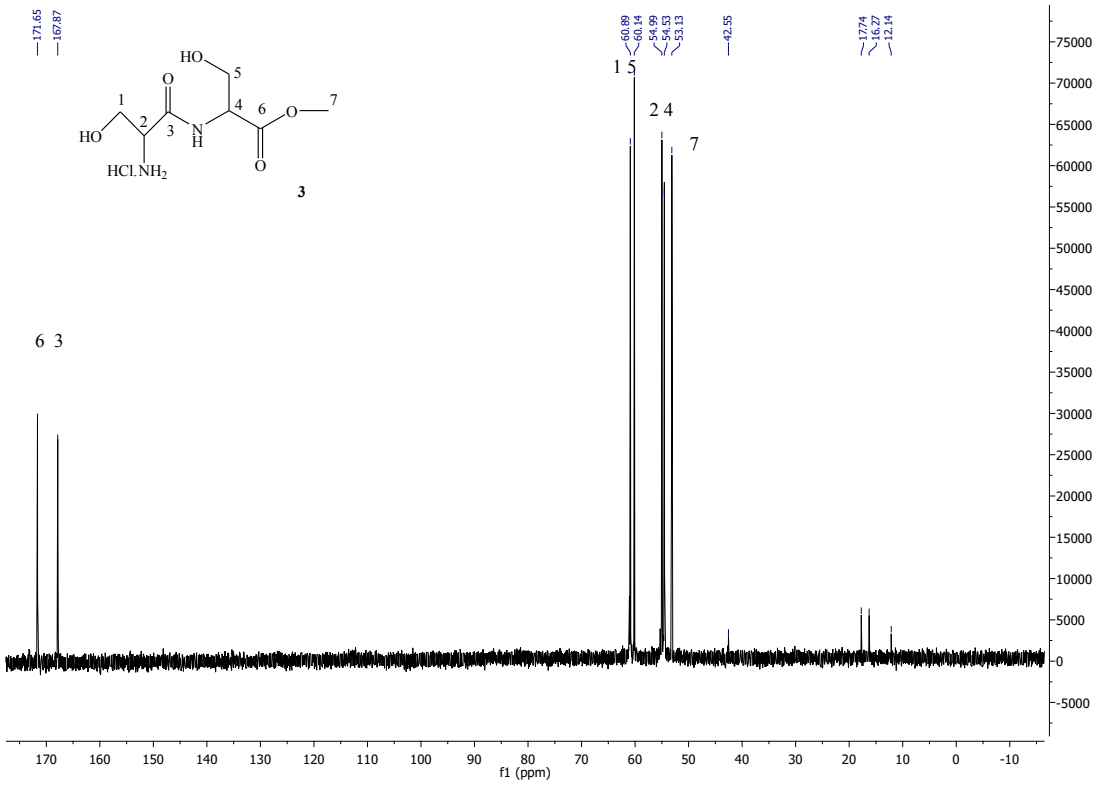
Appendix 4: ^{13}C NMR N-Boc-Ser(O-TBS)-Ser-OMe (2).

* Peaks at 14, 22, and 31 ppm due to hexane solvent

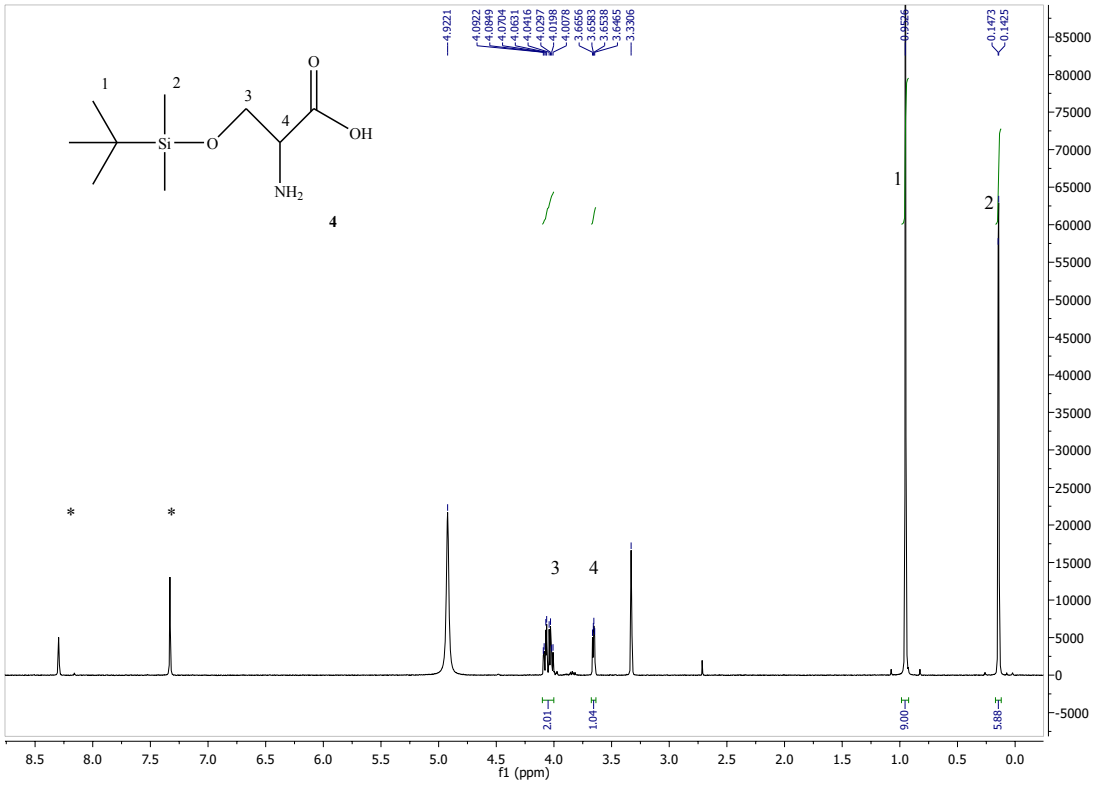


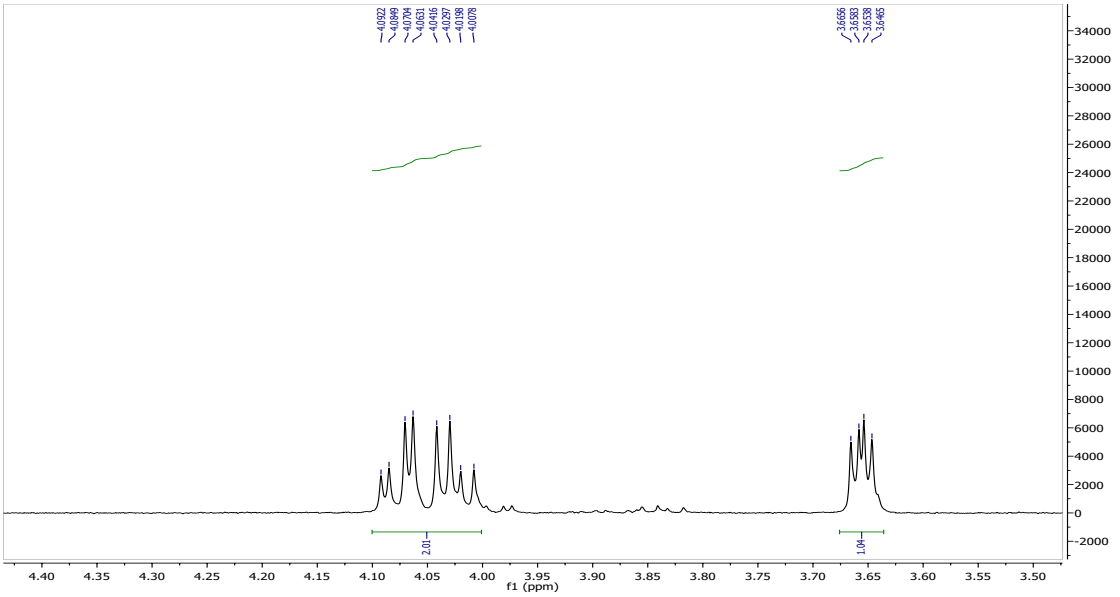
Appendix 5: ^{1}H NMR H_2N-Ser-OH-Ser-OMe (3).





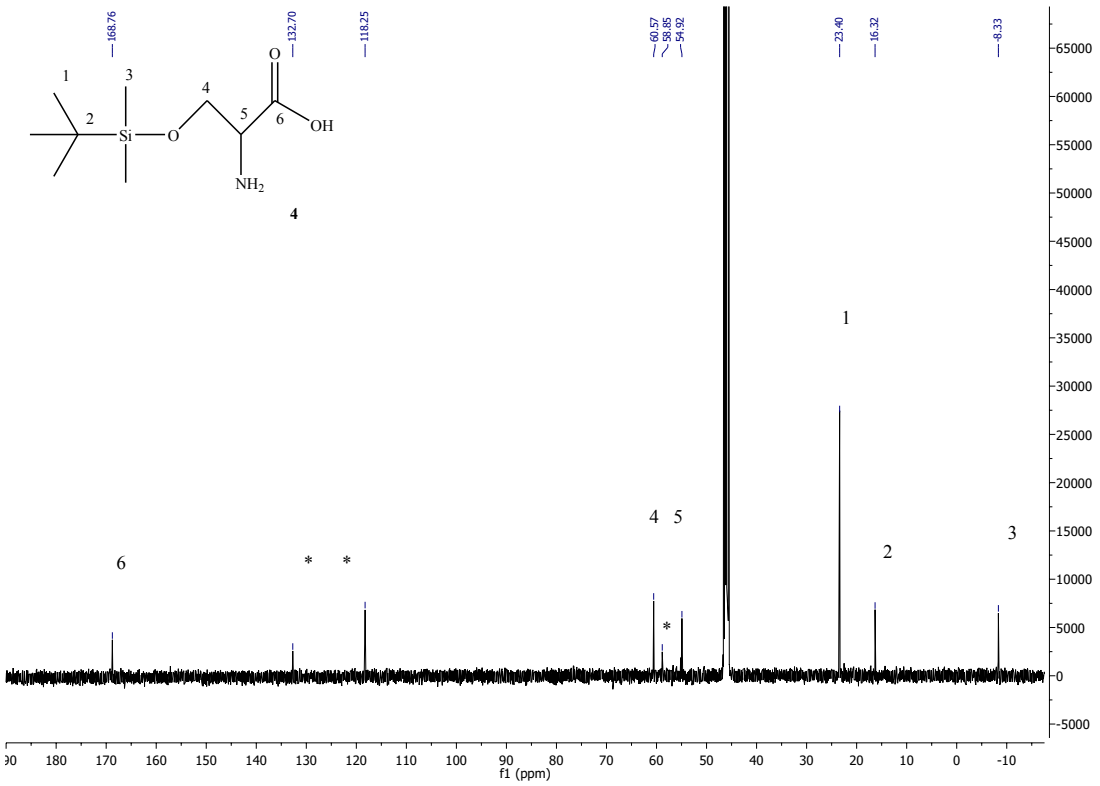
Appendix 6: ^{13}C NMR H_2N-Ser-OH-Ser-OMe (3).





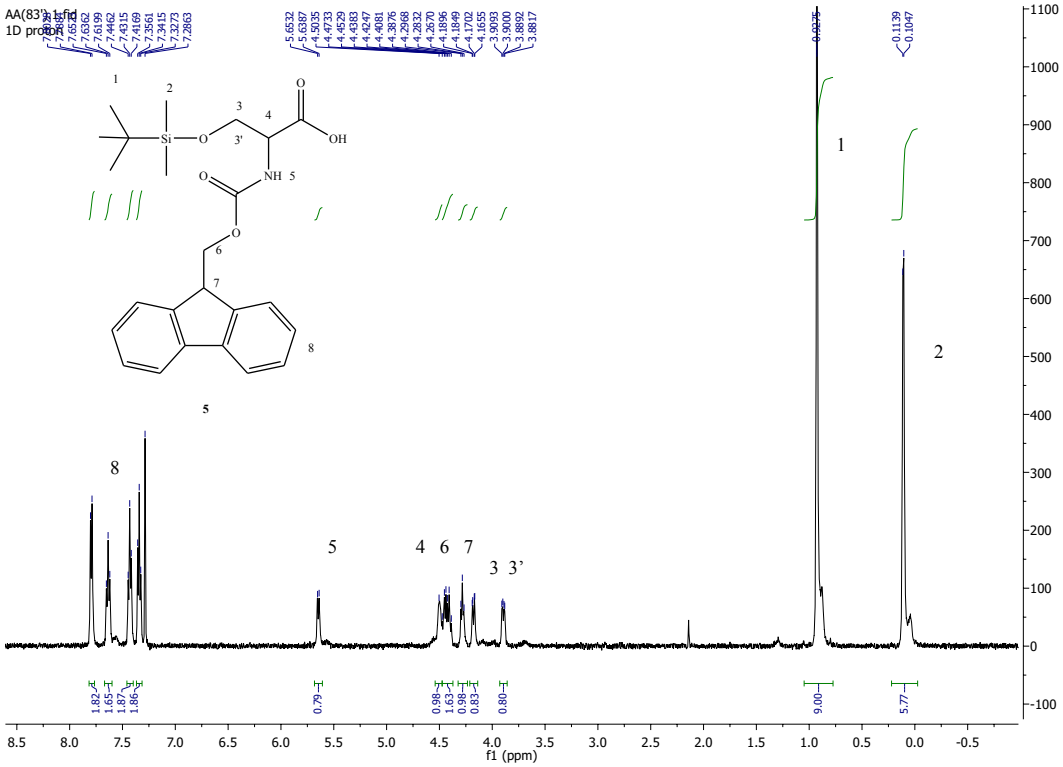
Appendix 7: ^{1}H NMR Ser-OH(O-TBS) (4).

* Peaks at 7.3 and 8.3 ppm due to impurities from the reagent imidazole.

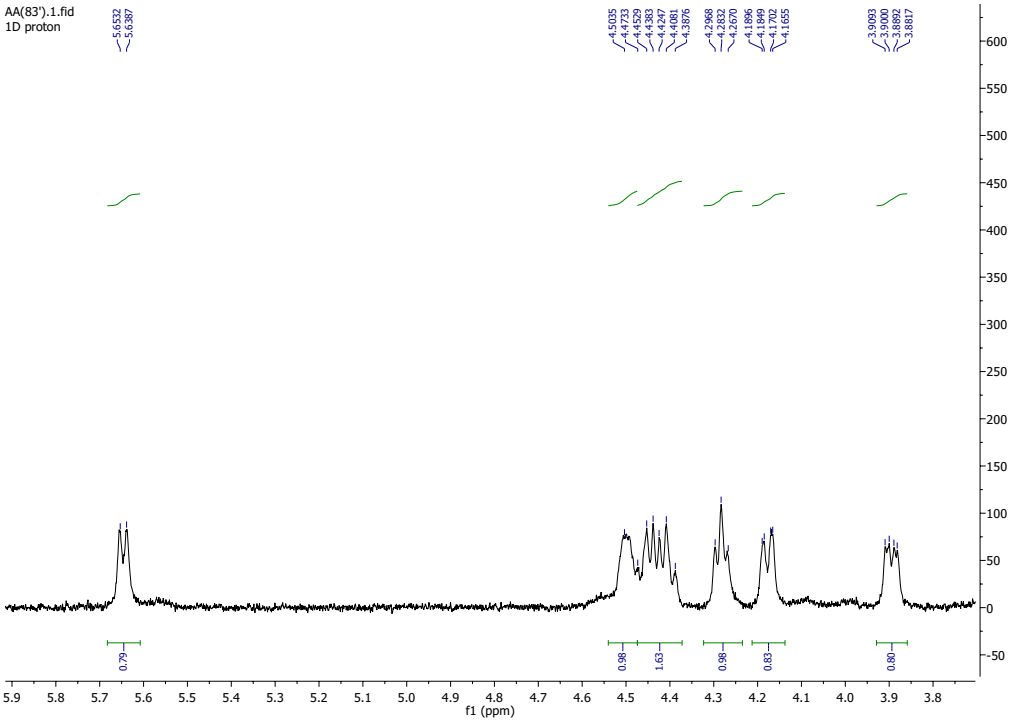


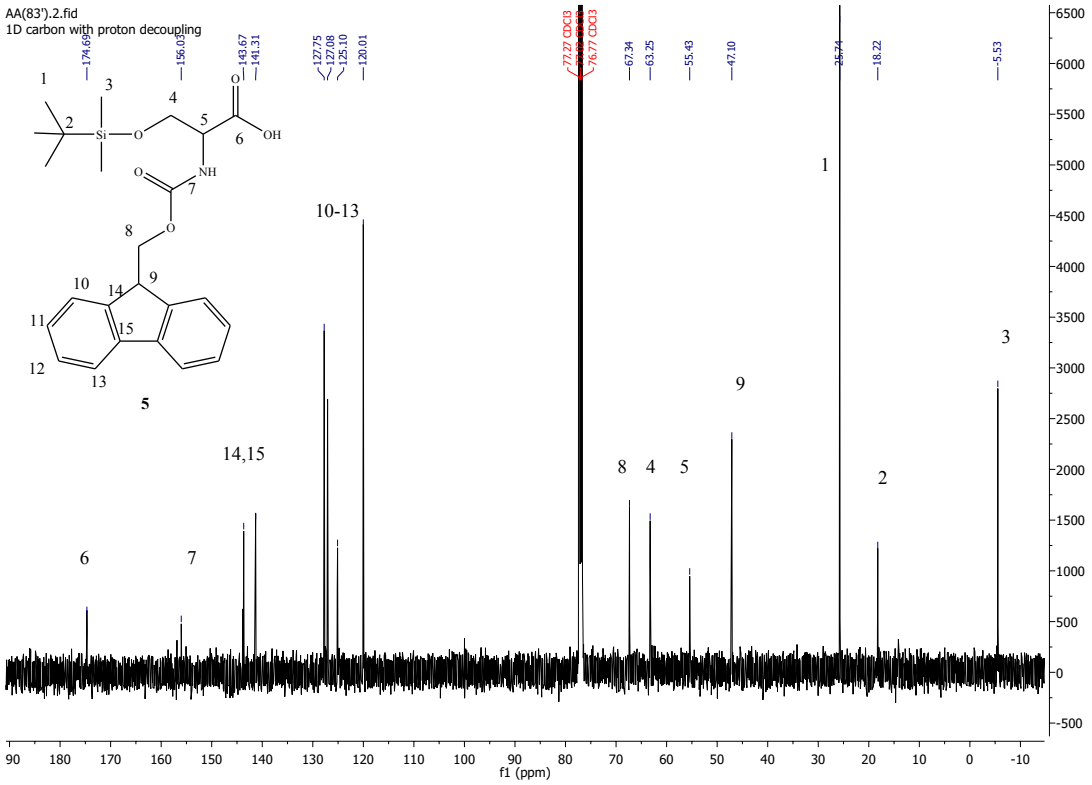
Appendix 8: ^{13}C NMR Ser-OH(O-TBS) (4).

* Impurities from the reagent imidazole

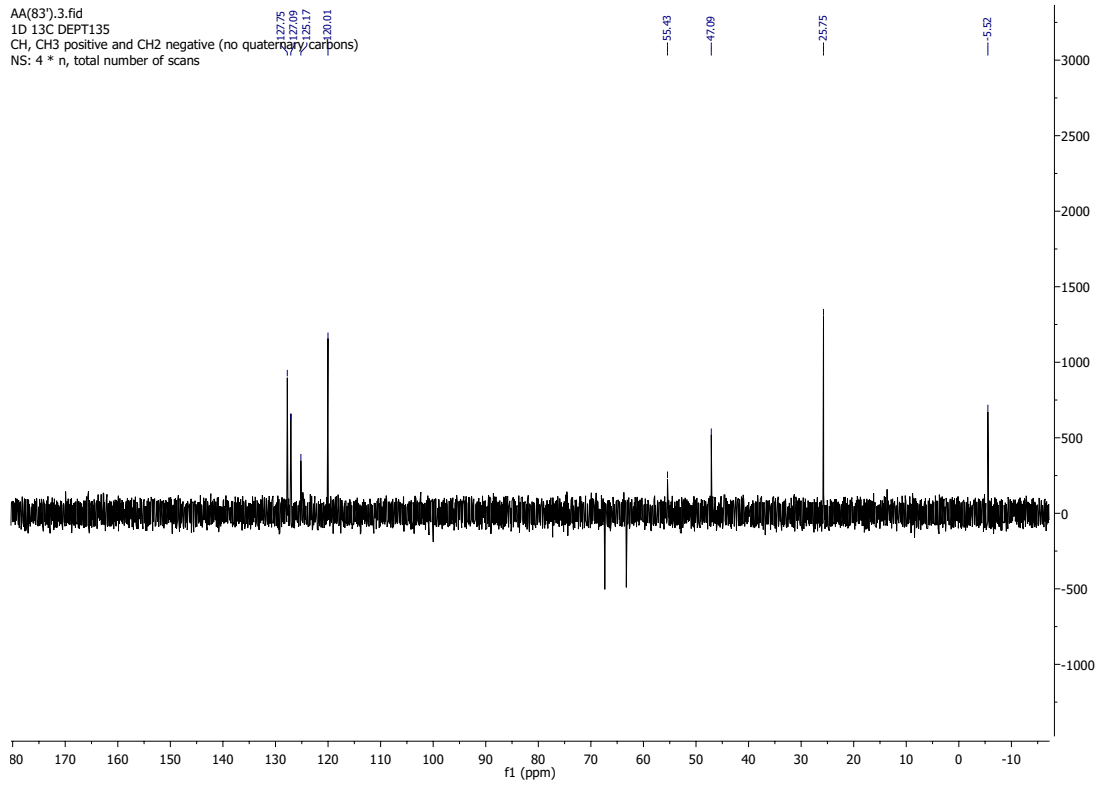


Appendix 9: ^{1}H NMR Fmoc-Ser-OH(O-TBS) (5).

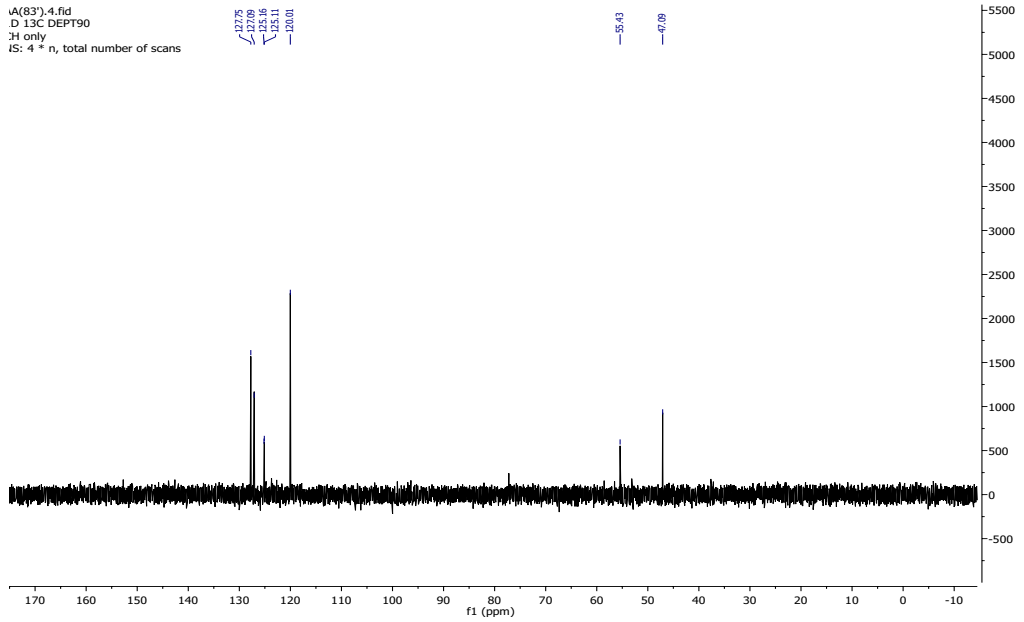




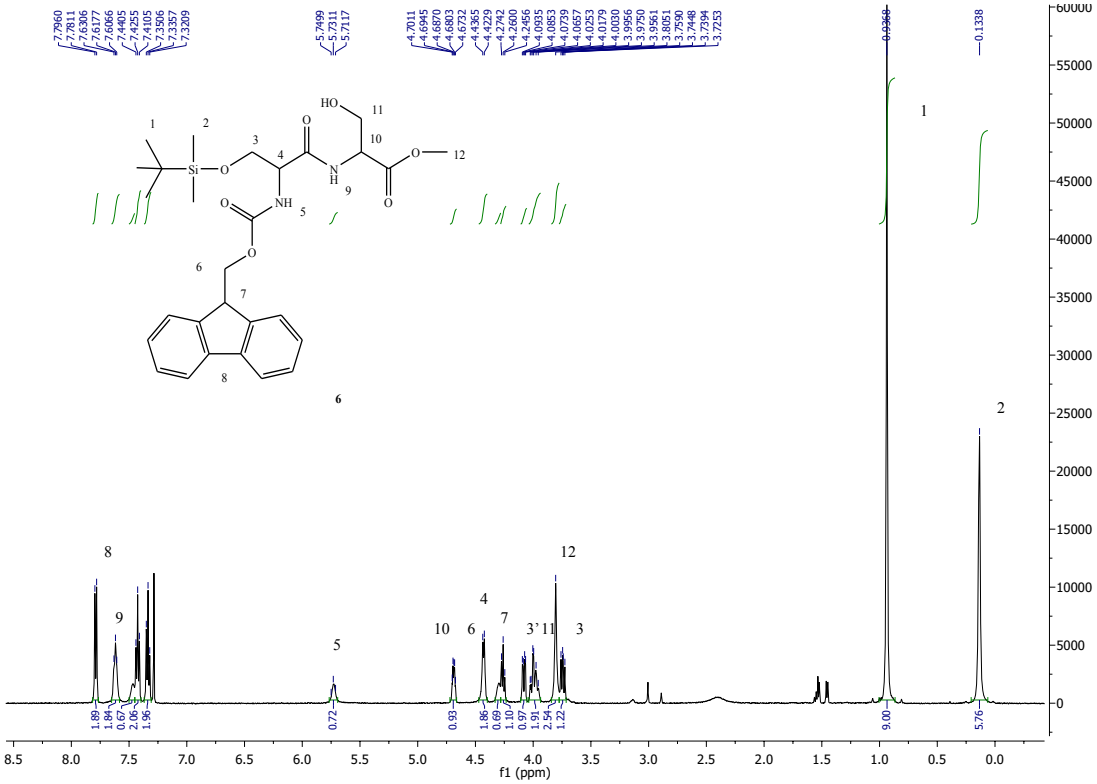
Appendix 10: ^{13}C NMR Fmoc-Ser-OH(O-TBS) (5).



Appendix 11: DEPT 135 NMR Fmoc-Ser-OH(O-TBS) (5).



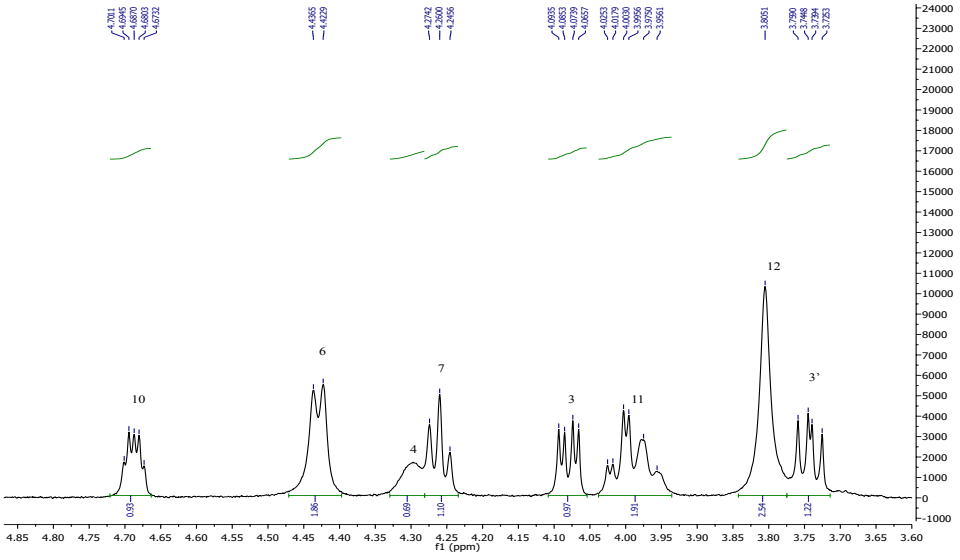
Appendix 12: DEPT 90 NMR Fmoc-Ser-OH(O-TBS) (5).

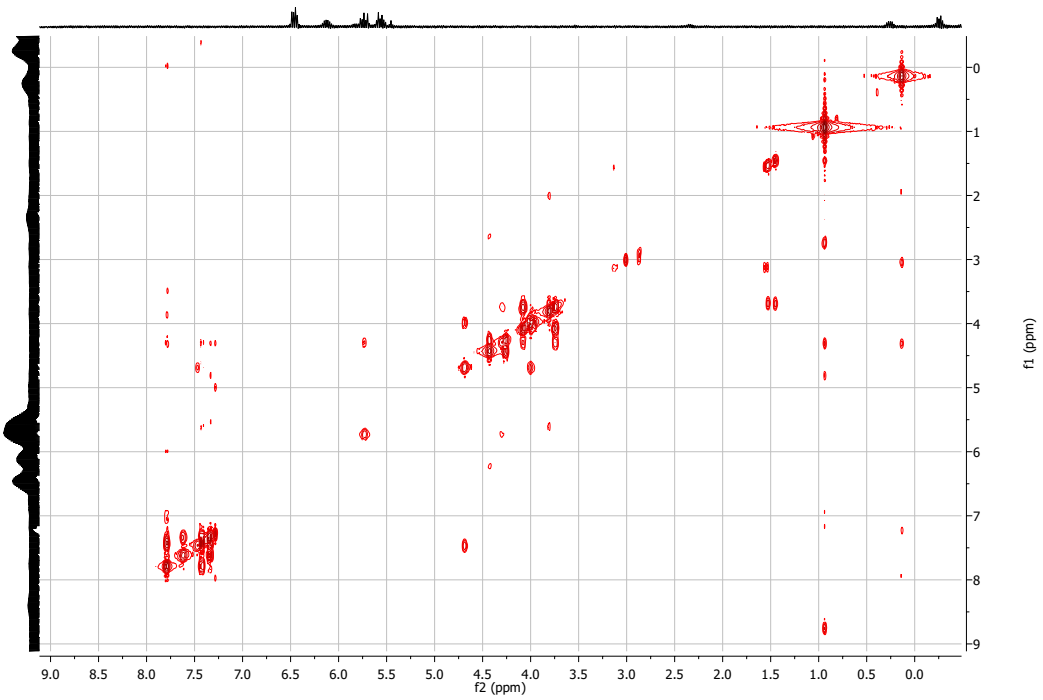


Appendix 13: ^{1}H NMR Fmoc-Ser(O-TBS)-Ser-OMe (6).

* Peaks at 1.2 ppm due to EtOAc solvent

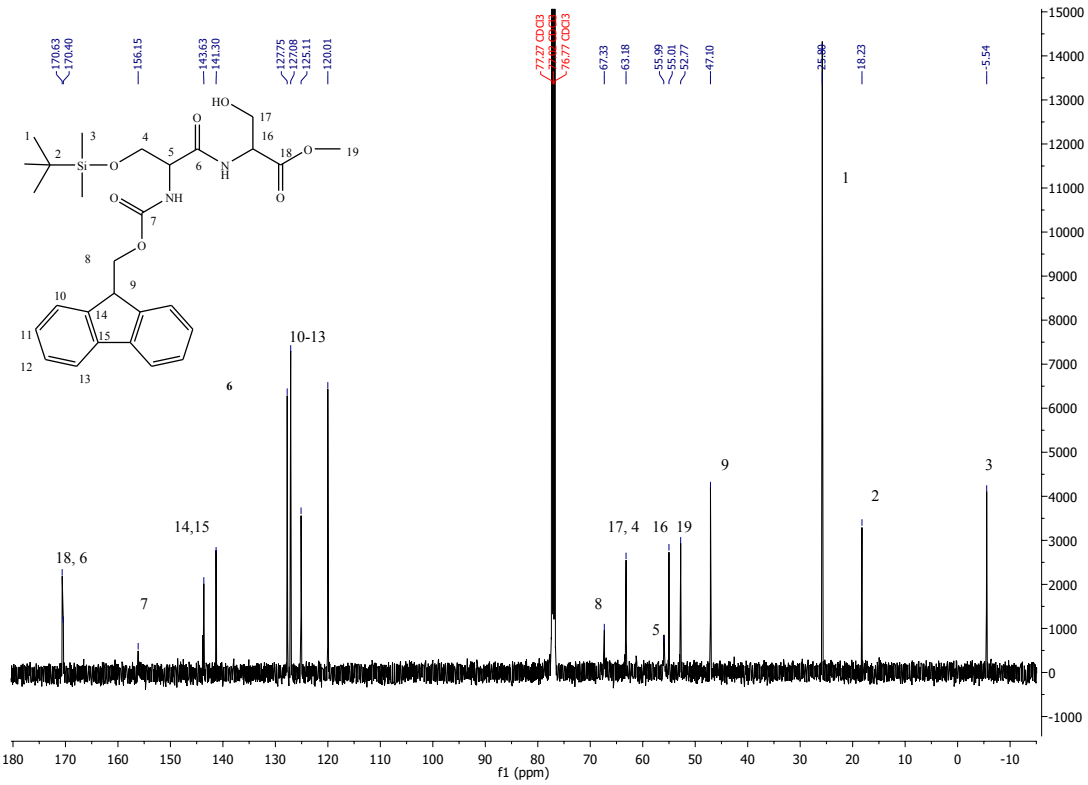
The singlet peak at 3.8 ppm represents the methyl ester group (OCH_3) and CH_2 and CH as multiplets at 4.04-3.9 ppm and 4.69 ppm respectively from the serine methyl ester.





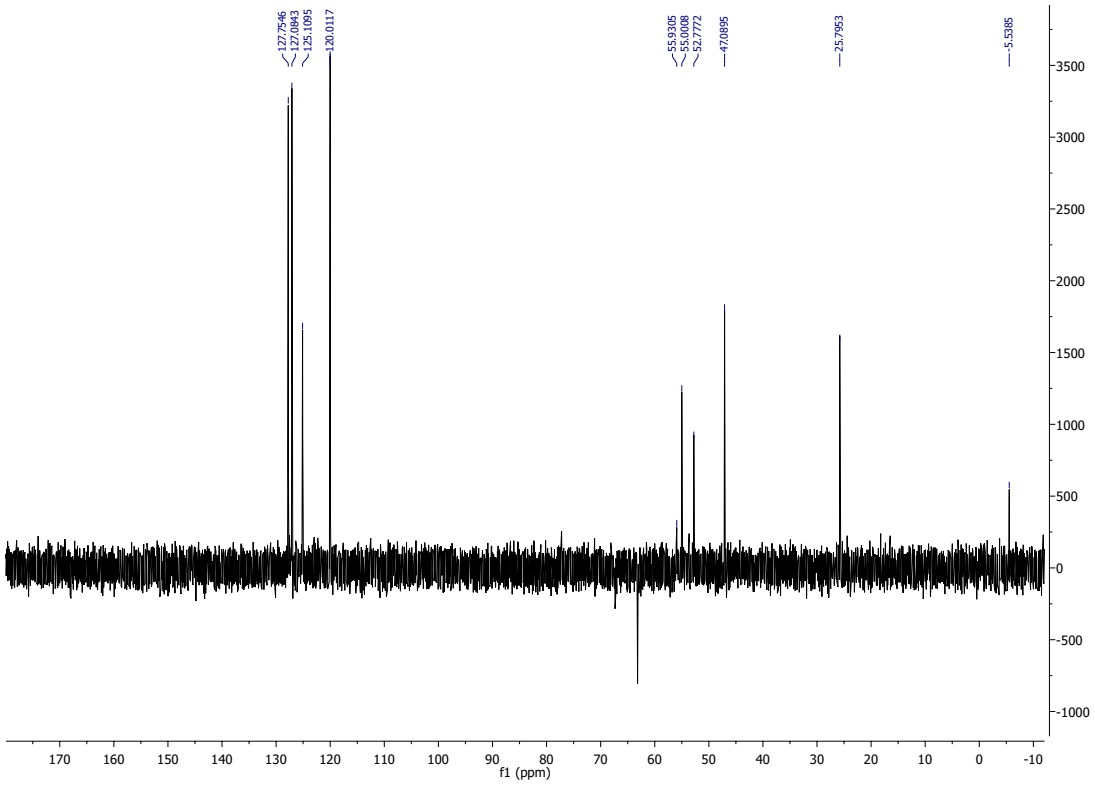
Appendix 14: COSY NMR Fmoc-Ser(O-TBS)-Ser-OMe (6).

2-Dimension NMR correlation spectroscopy (COSY) of proton-proton correlation analysis was done to identify the coupling between neighbouring protons. The square pattern represents the coupling between those specific peaks from the 1D proton NMR spectra on the X and Y axis of the 2D matrix.

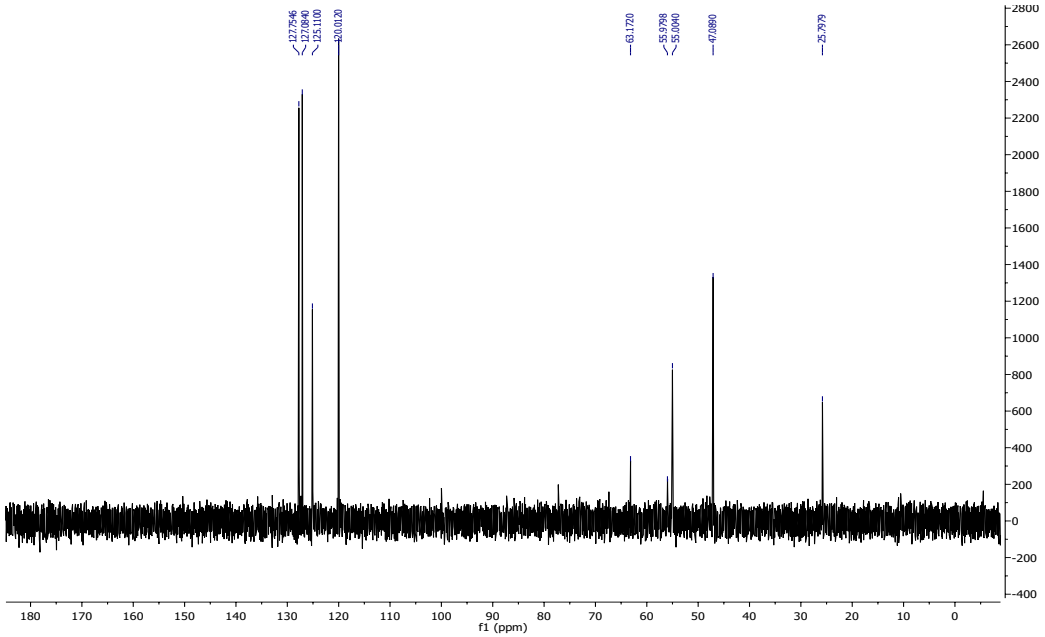


Appendix 15: ^{13}C NMR Fmoc-Ser(O-TBS)-Ser-OMe (6).

Distortionless enhancement by polarization transfer (DEPT) 135 and DEPT 90 was done to identify carbon peaks. DEPT determine the number of hydrogens attached to each carbon in a compound. DEPT 135 yields CH and CH_3 as positive peaks whereas CH_2 as negative signals. DEPT 90 gives only CH peaks and quaternary carbon peaks are disappeared in both DEPT 135 and DEPT 90.

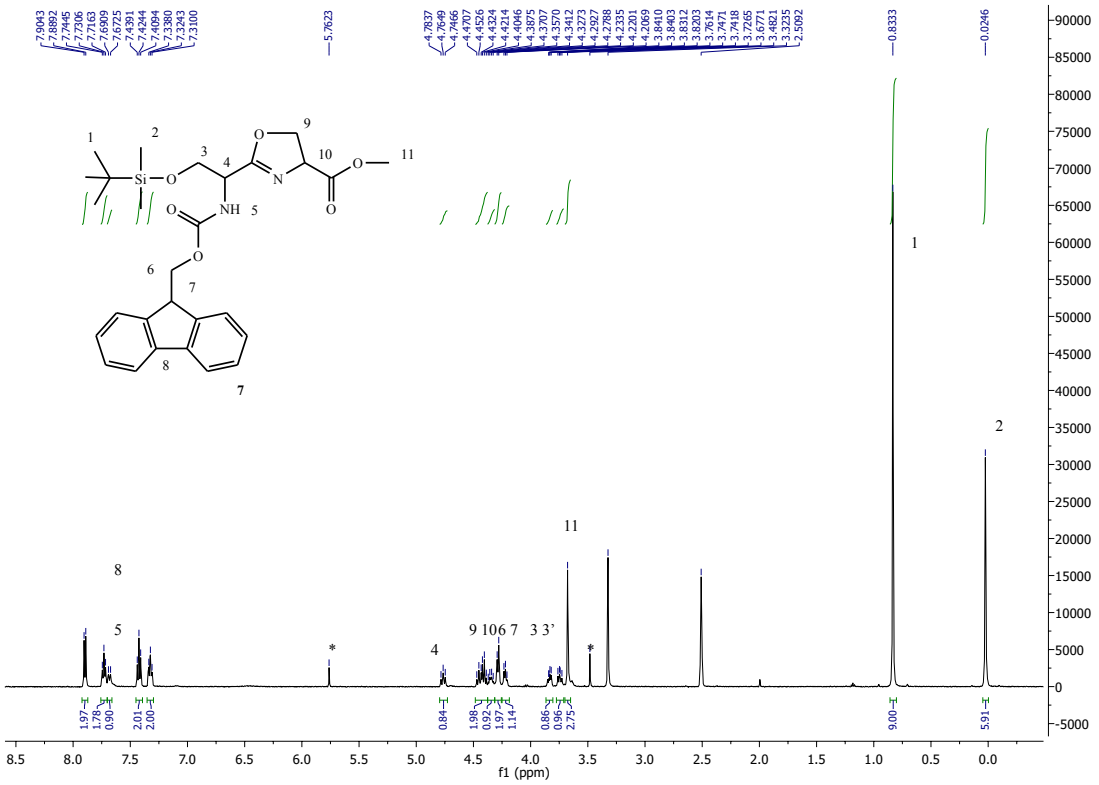


Appendix 16: DEPT 135 NMR Fmoc-Ser(O-TBS)-Ser-OMe (6).



Appendix 17: DEPT 90 NMR Fmoc-Ser(O-TBS)-Ser-OMe (6).

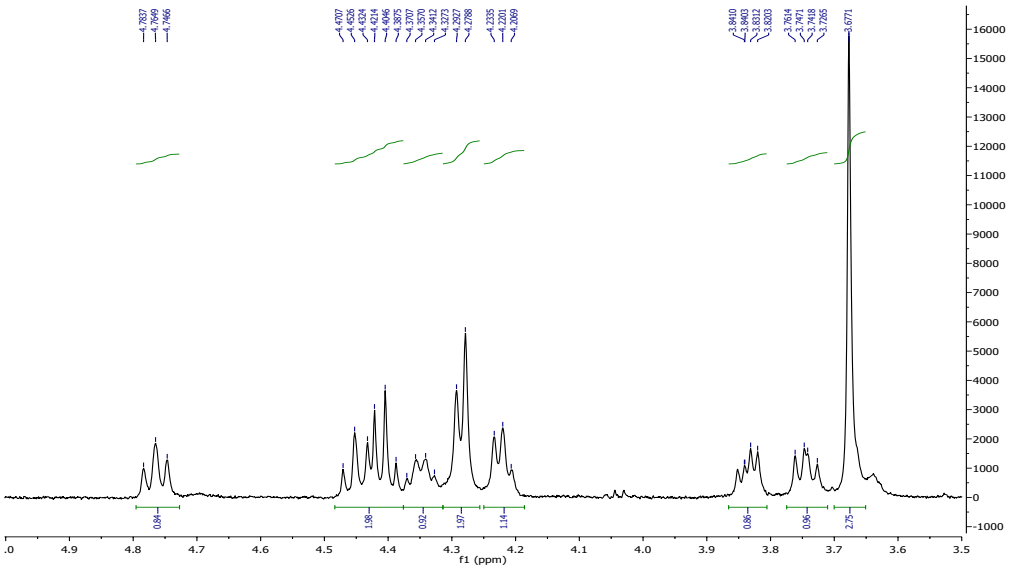
The presence of small peak for the CH_3 single at 25 ppm in the DEPT 90 spectrum that should zero intensity instead because of the leakage that can happen. Leakage can occur from incorrectly calibrated pulse width for the spectrometer.

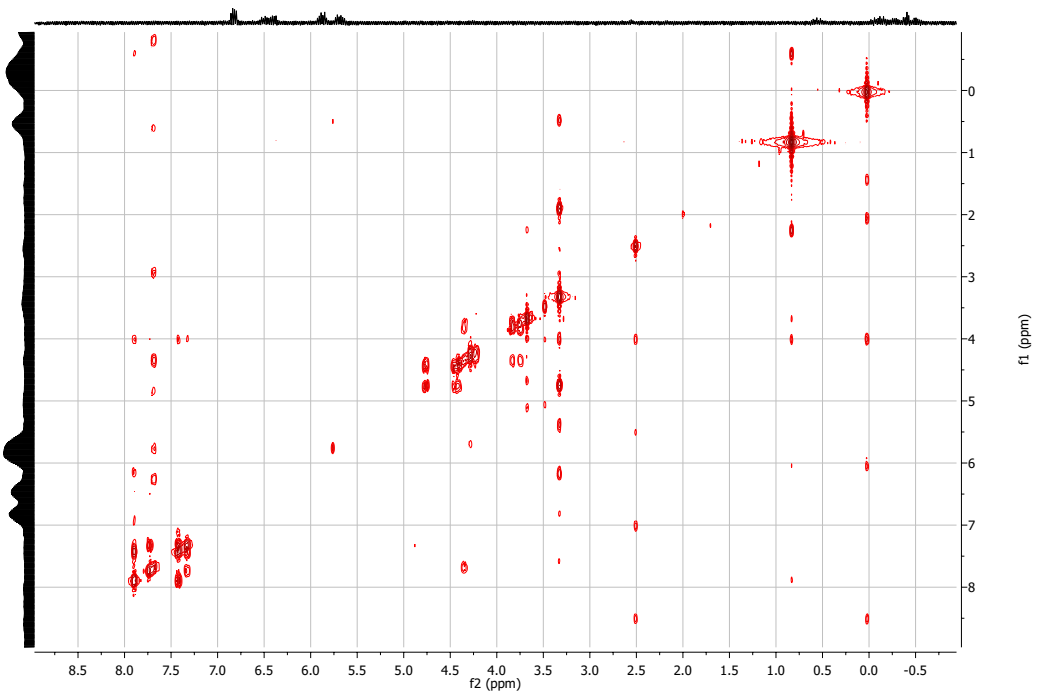


Appendix 18: ^{1}H NMR Fmoc-oxazoline-OMe (7).

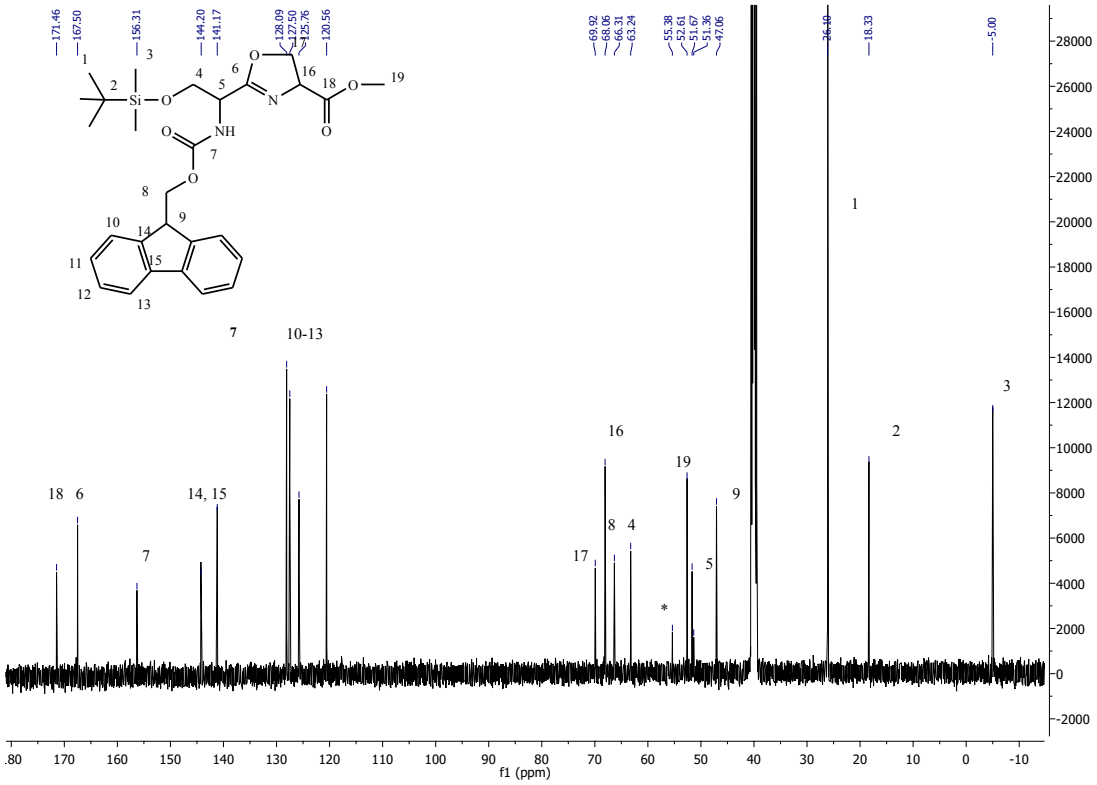
* Peak at 3.4 ppm due to the by-product from Burgess reagent and it is 55 ppm in 13C

* Peak at 5.6 due to DCM solvent



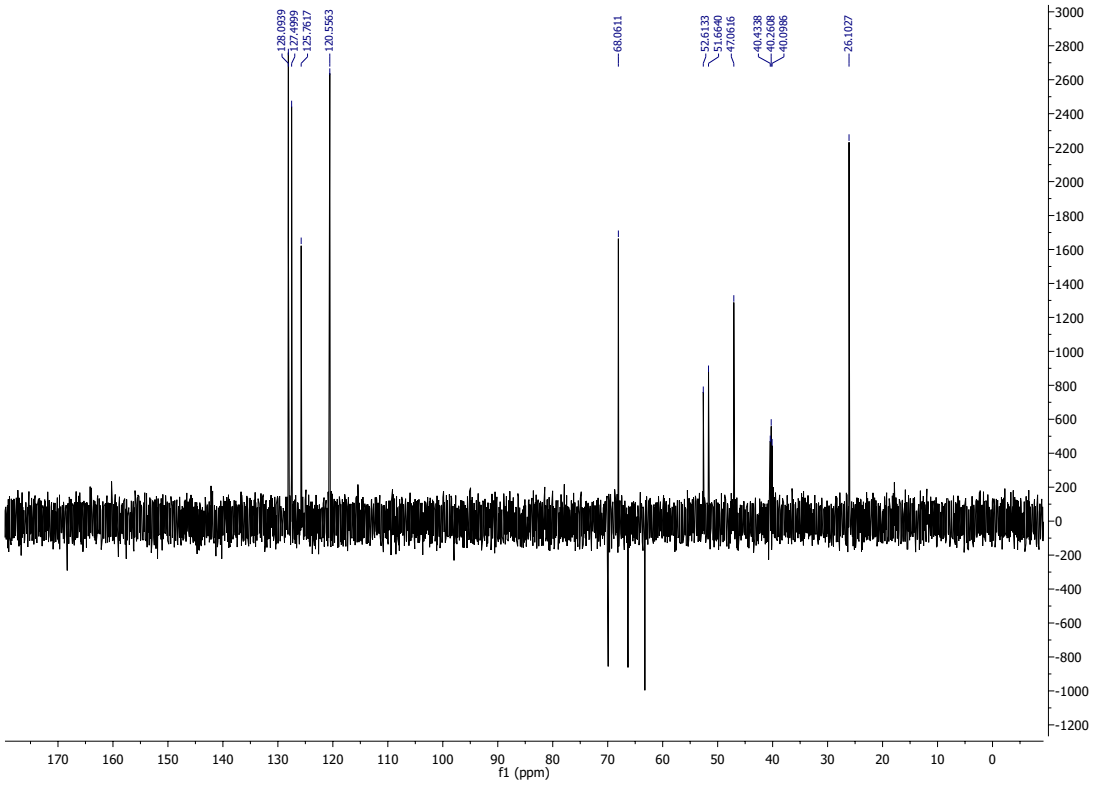


Appendix 19: COSY NMR Fmoc-oxazoline-OMe (7).

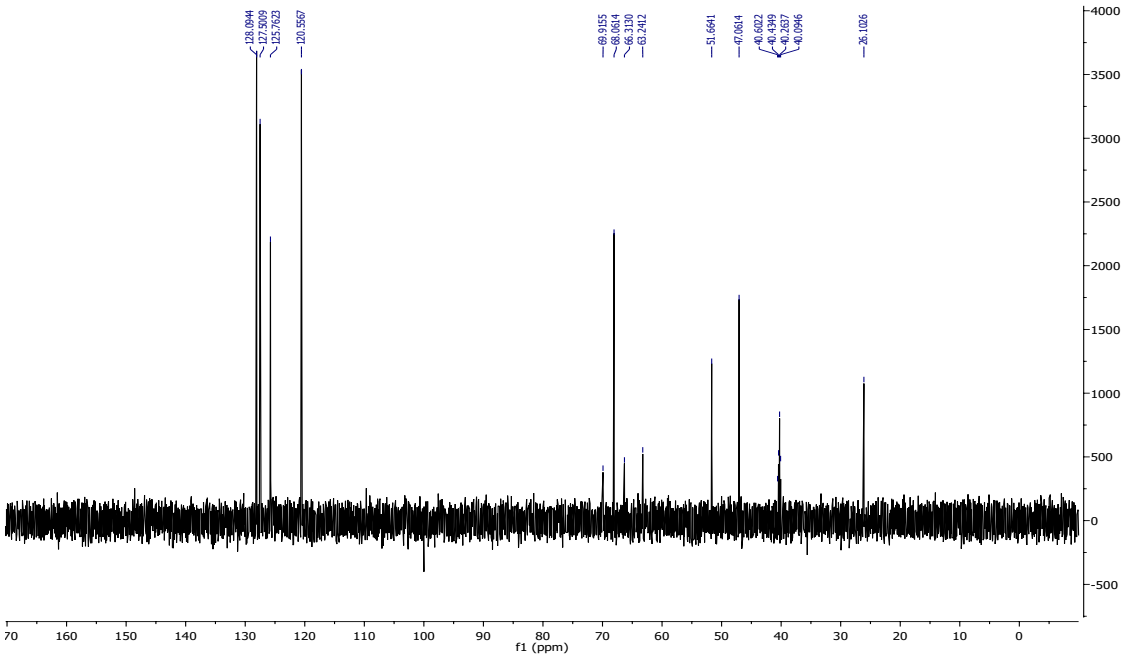


Appendix 20: ^{13}C NMR Fmoc-oxazoline-OMe (7).

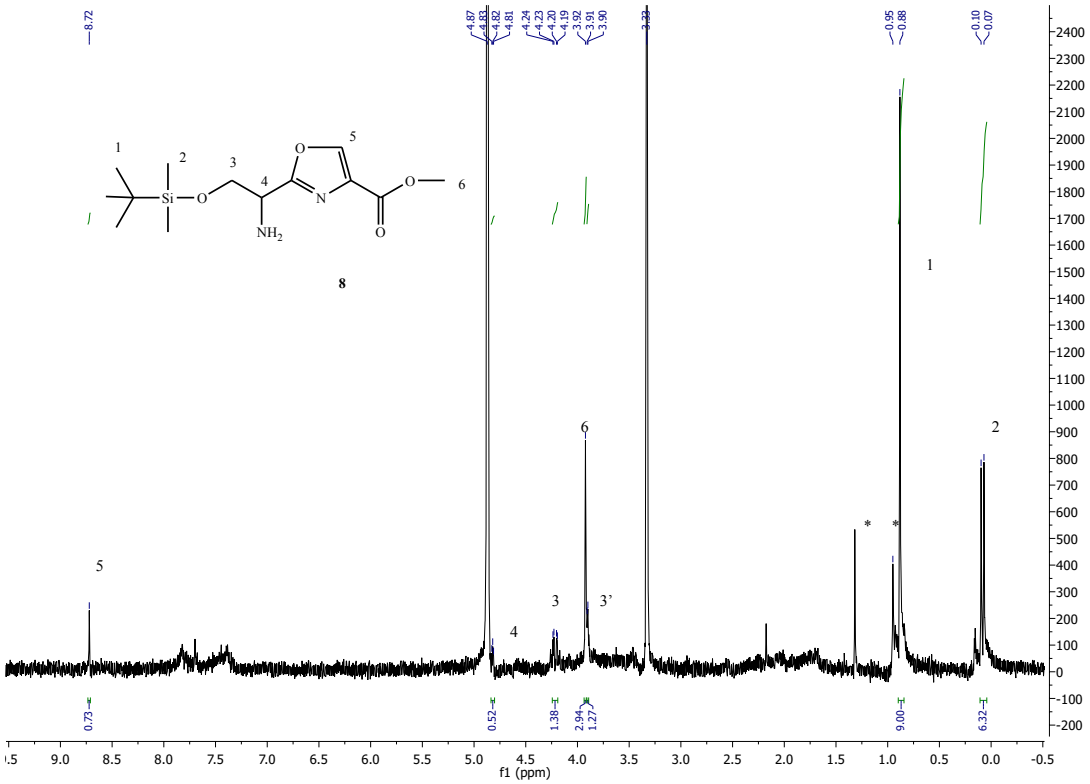
* Peak at 55 ppm due to by-product from Burgess reagent



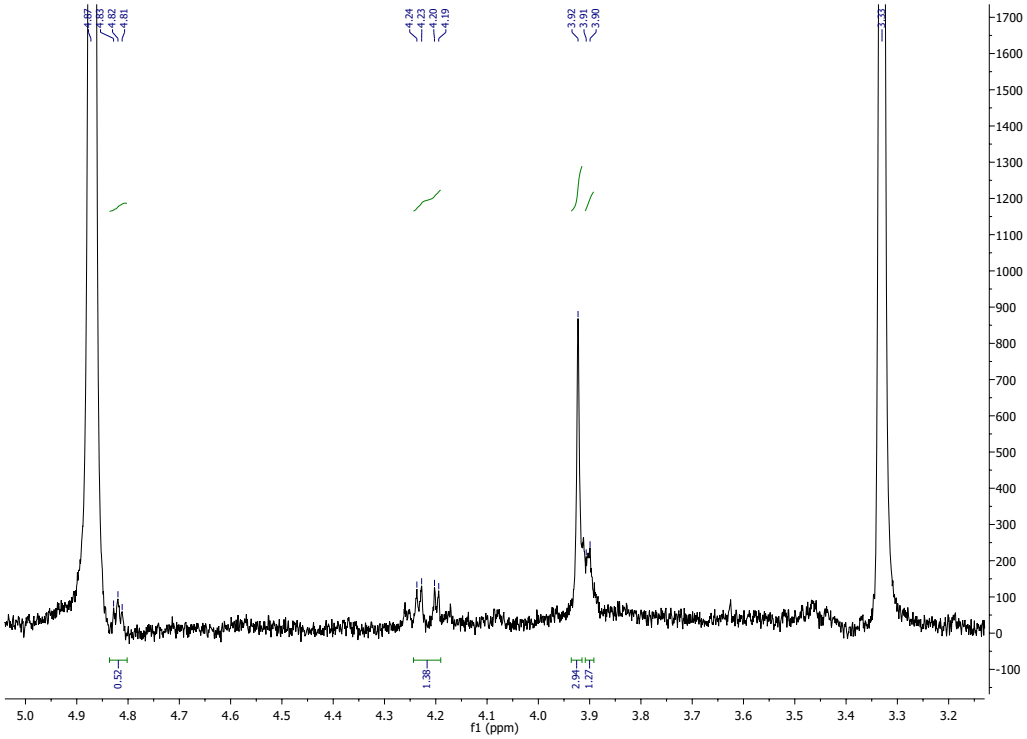
Appendix 21: DEPT 135 NMR Fmoc-oxazoline-OMe (7).



Appendix 22: DEPT 90 NMR Fmoc-oxazoline-OMe (7).

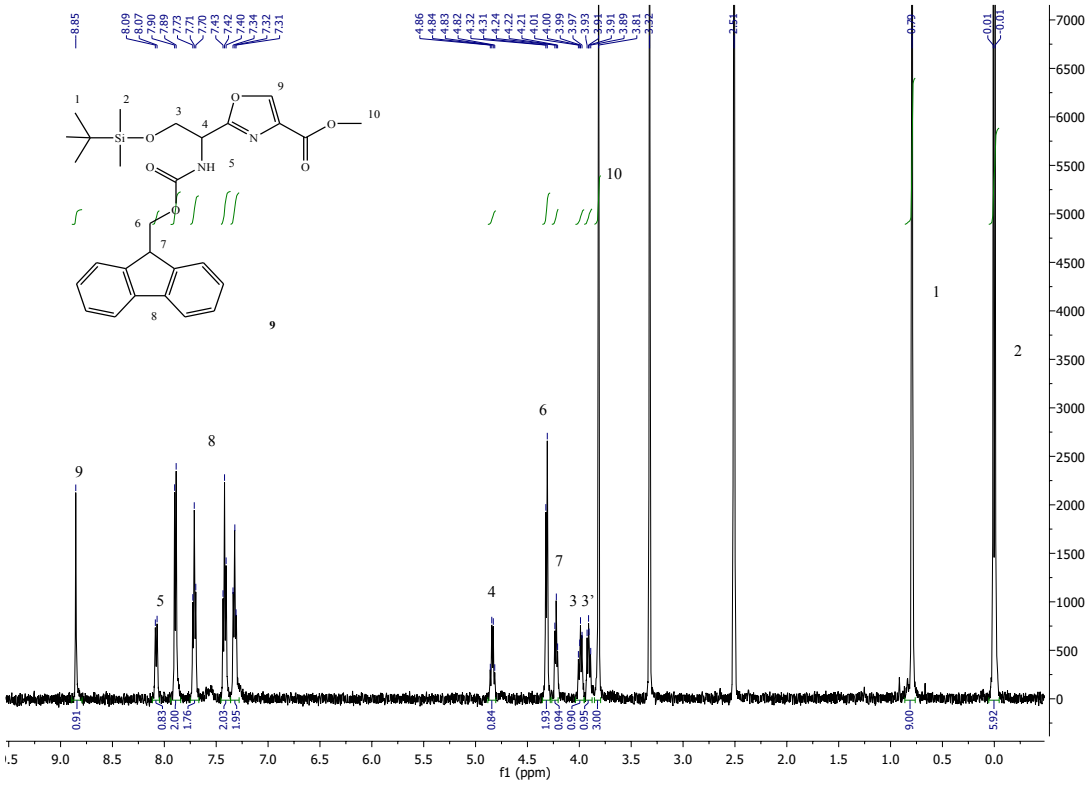


Appendix 23: ^{1}H NMR H_2N-oxazole-OMe (8).

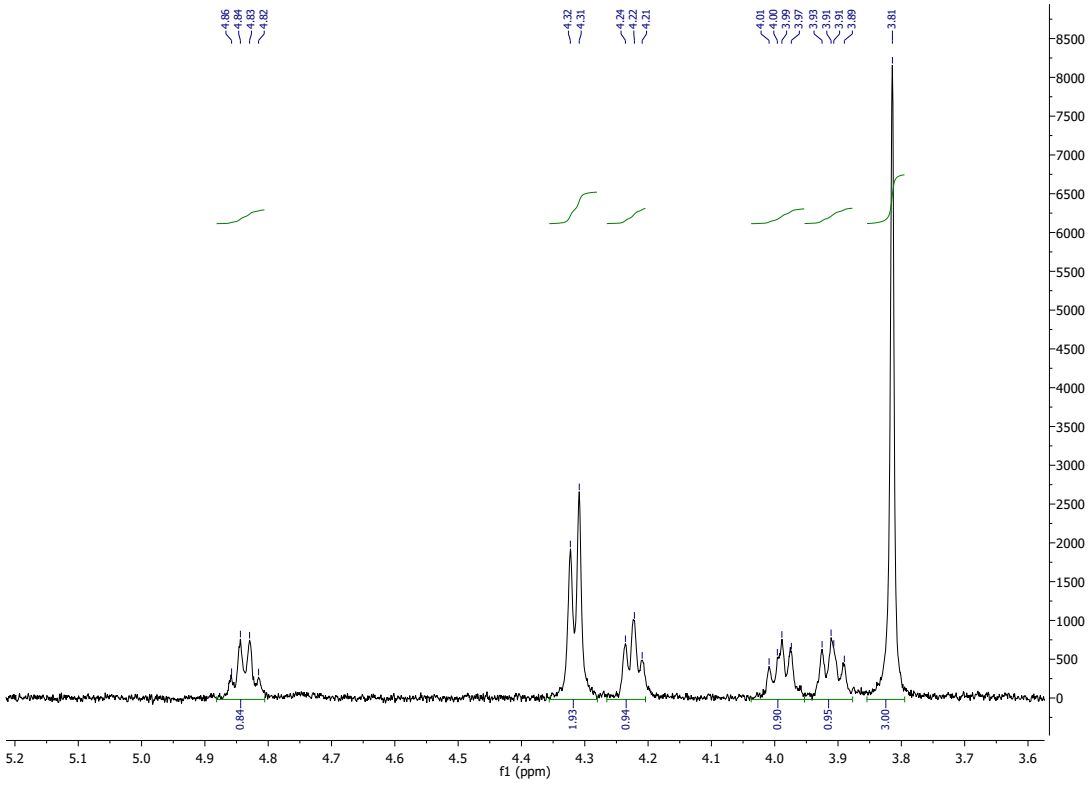


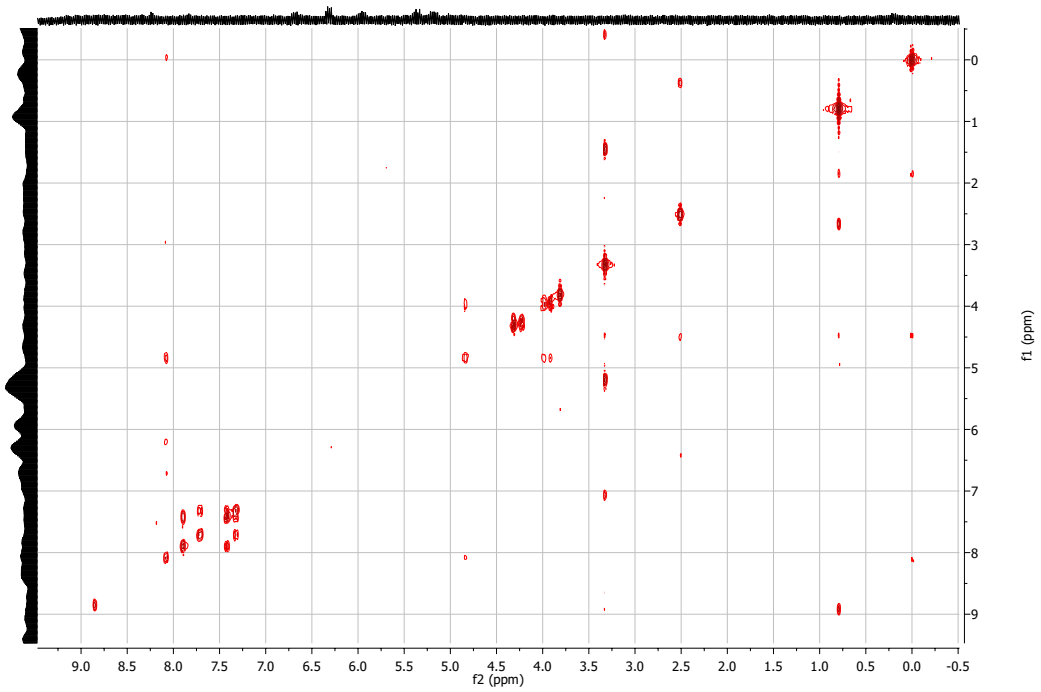
* Peaks at 0.99 and 1.32 ppm for the hexane solvent and 2.1 ppm for acetone solvent.

The ^{1}H NMR data shows the expected signal of the aromatic proton at δ 8.72 ppm (s, 1H; OCH=C) that confirmed the formation of oxazole ring. Also, it confirmed the deprotection of the Fmoc group by the disappearance of the Fmoc protons.



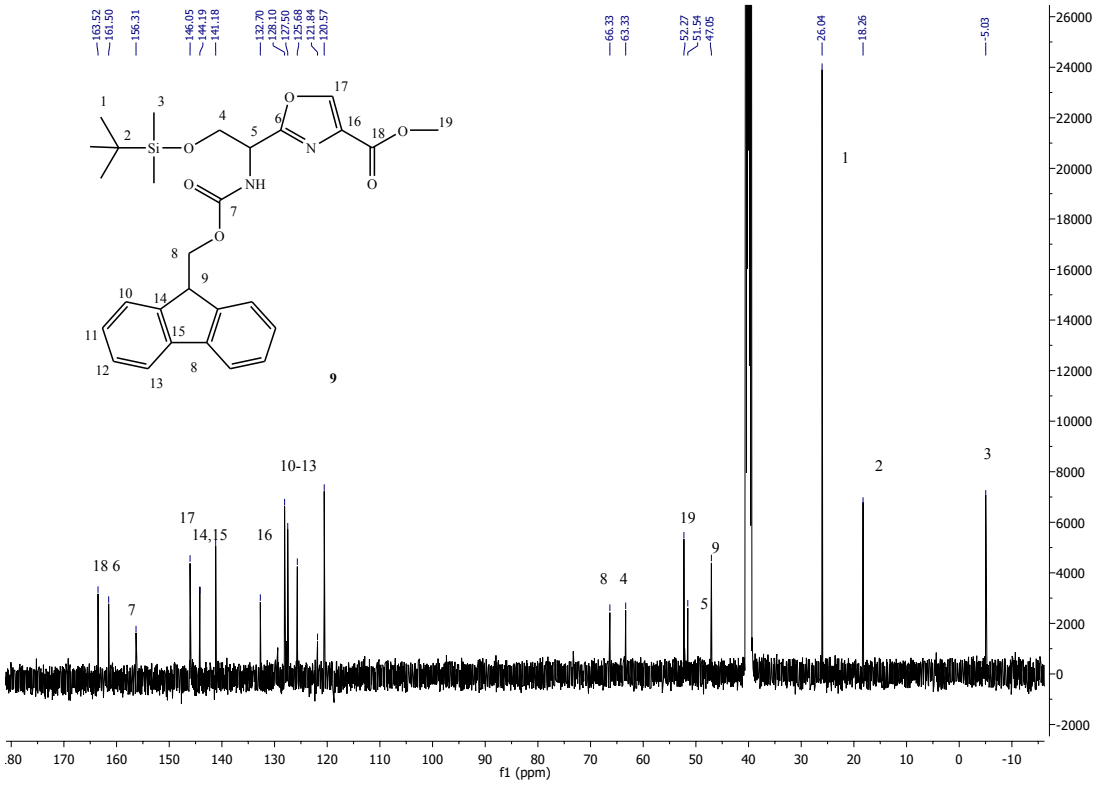
Appendix 24: ^{1}H NMR Fmoc-oxazole-OMe (9).



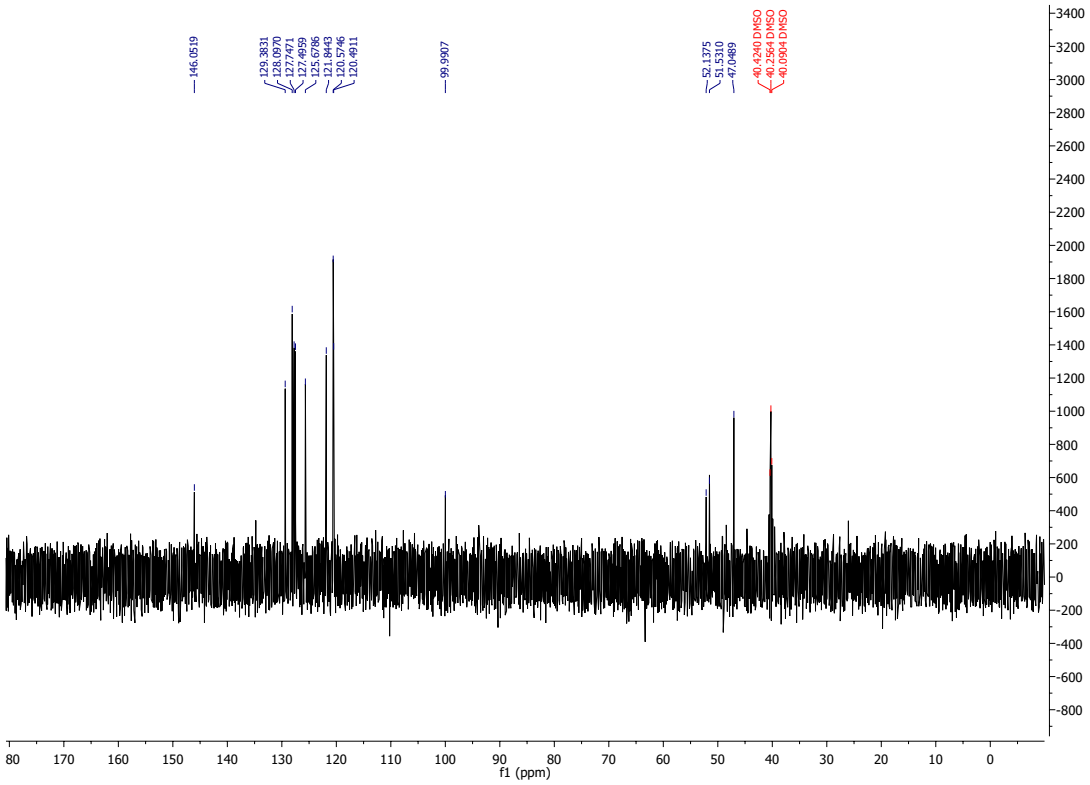


Appendix 25: COSY NMR Fmoc-oxazole-OMe (9).

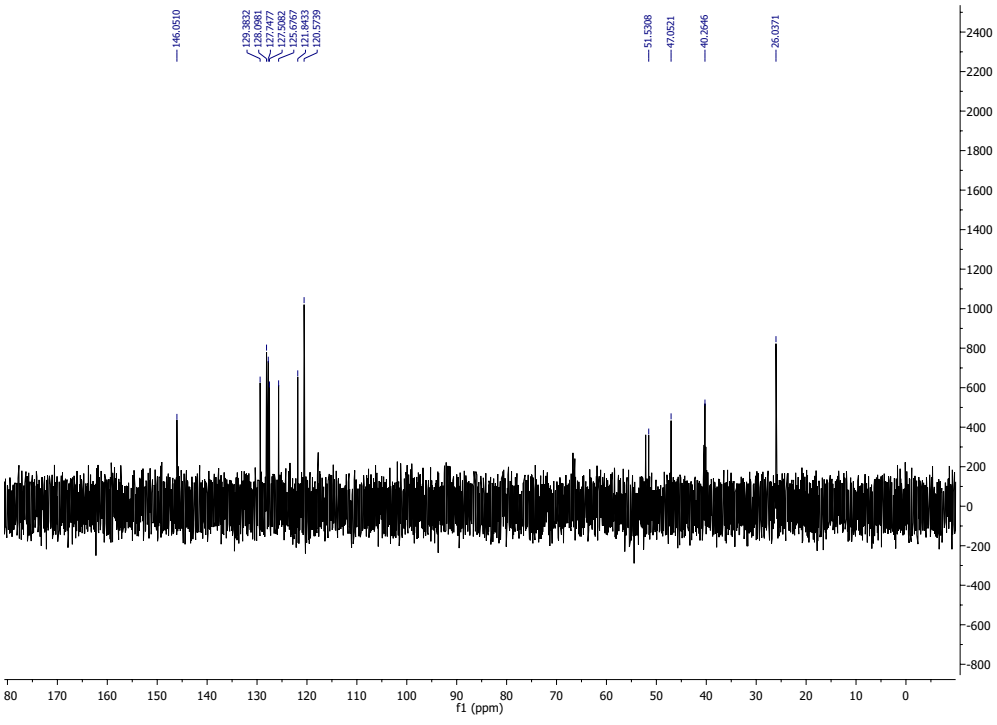
The signal appearing at δ 8.85 ppm (s, 1H; CH (oxazole)) in the 1H NMR spectrum revealed the formation of the oxazole ring



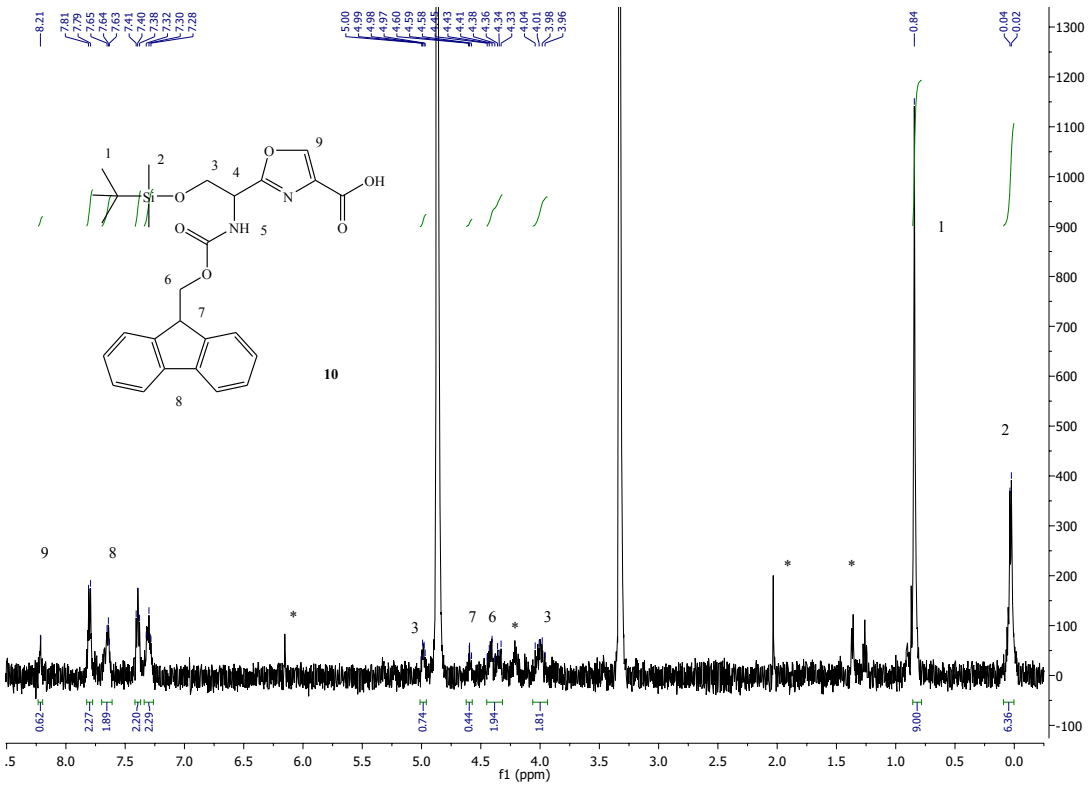
Appendix 26: ^{13}C NMR Fmoc-oxazole-OMe (9).



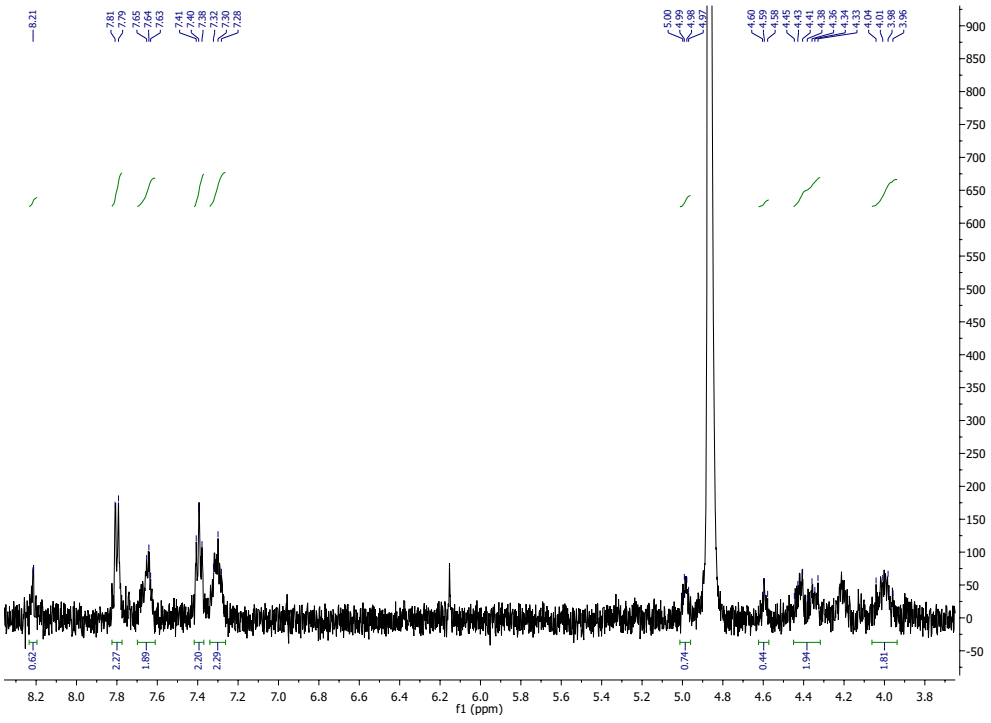
Appendix 27: DEPT 135 NMR Fmoc-oxazole-OMe (9).



Appendix 28: DEPT 90 NMR Fmoc-oxazole-OMe (9).

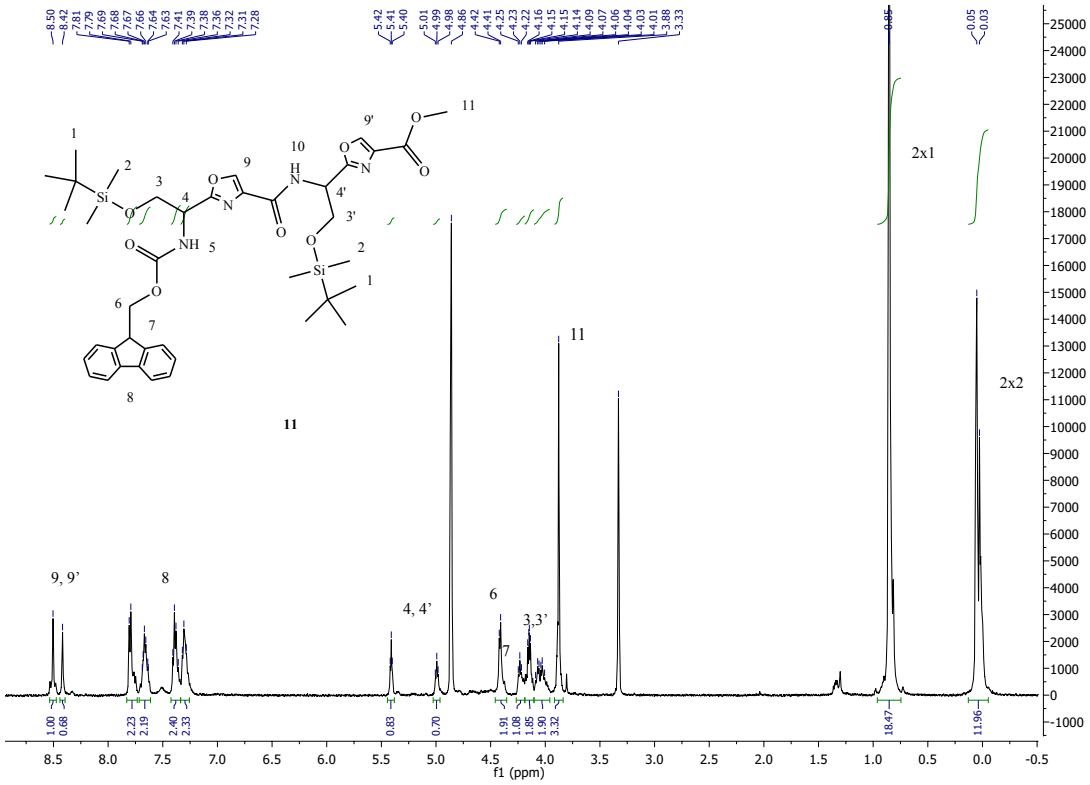


Appendix 29: ^{1}H NMR Fmoc-oxazole-COOH (10).

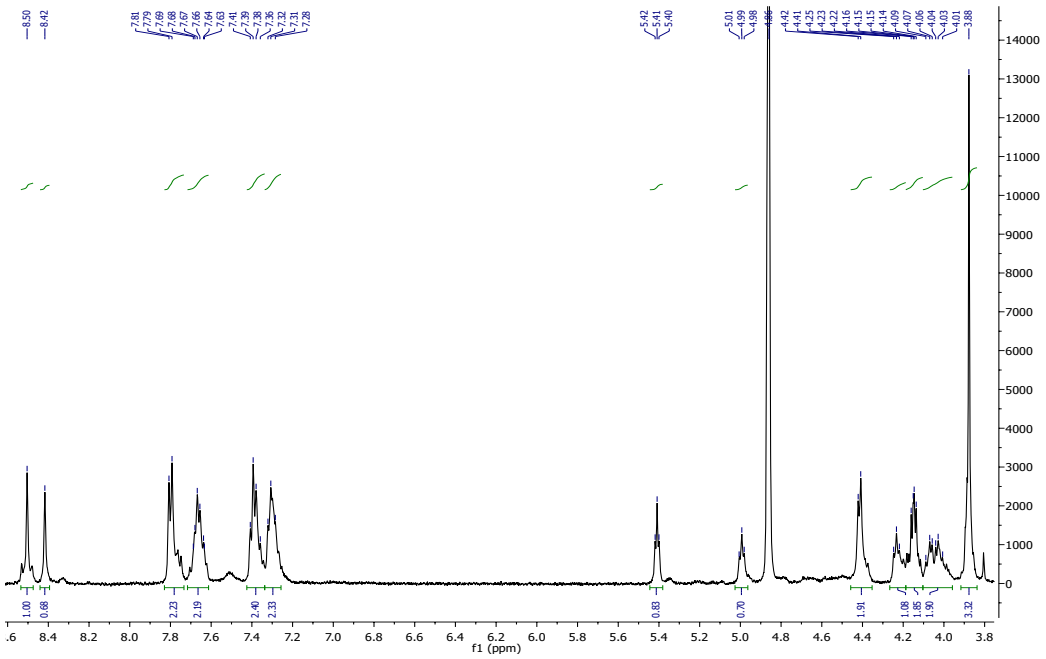


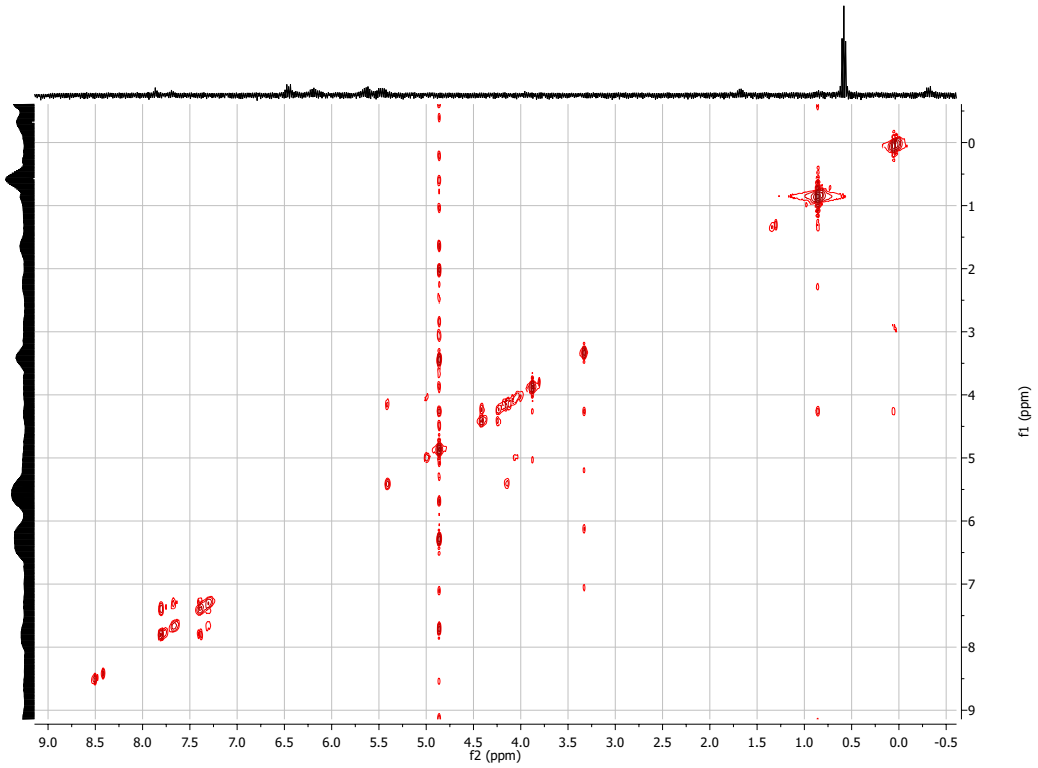
* Peaks at 1.24, 2.01 and 4.10 ppm due to EtOAc solvent.

The NMR spectrum of the deprotected Fmoc-oxazole-COOH was confirmed by the disappearance of the methyl ester proton that was at 3.81 ppm (s, OCH_3) in starting material Fmoc-oxazole-OMe.



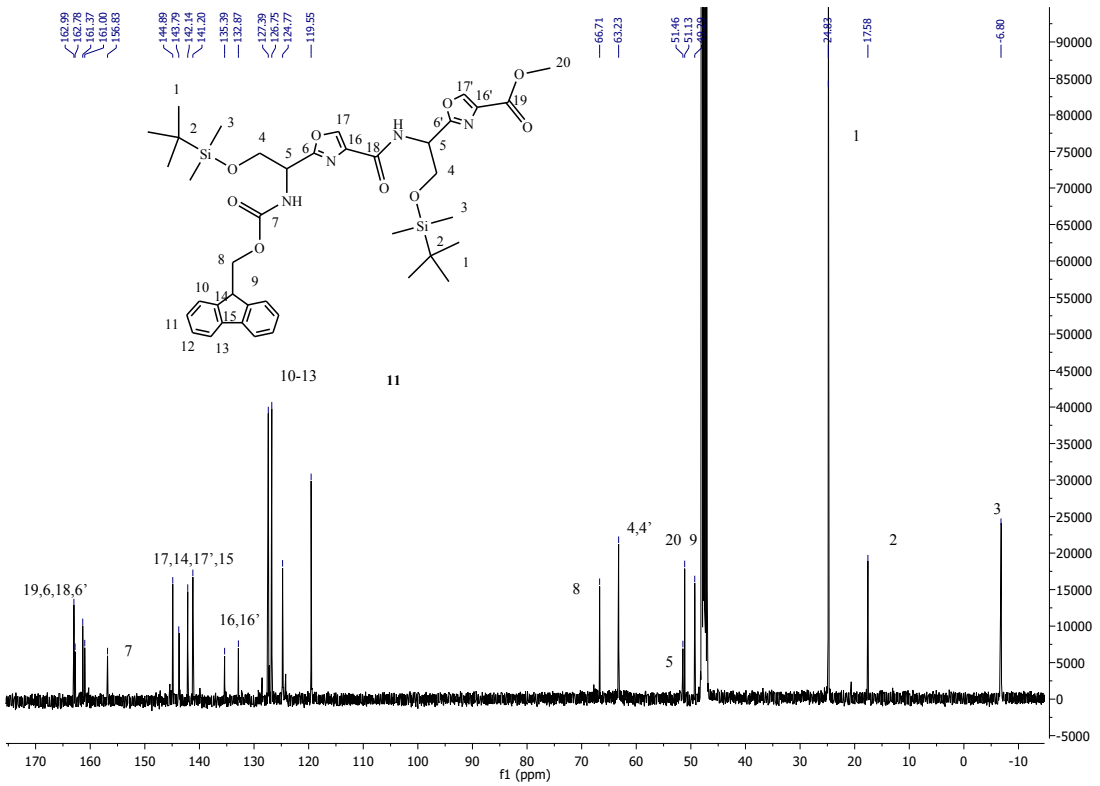
Appendix 30: ^{1}H NMR Fmoc-dioxazole-OMe (11).



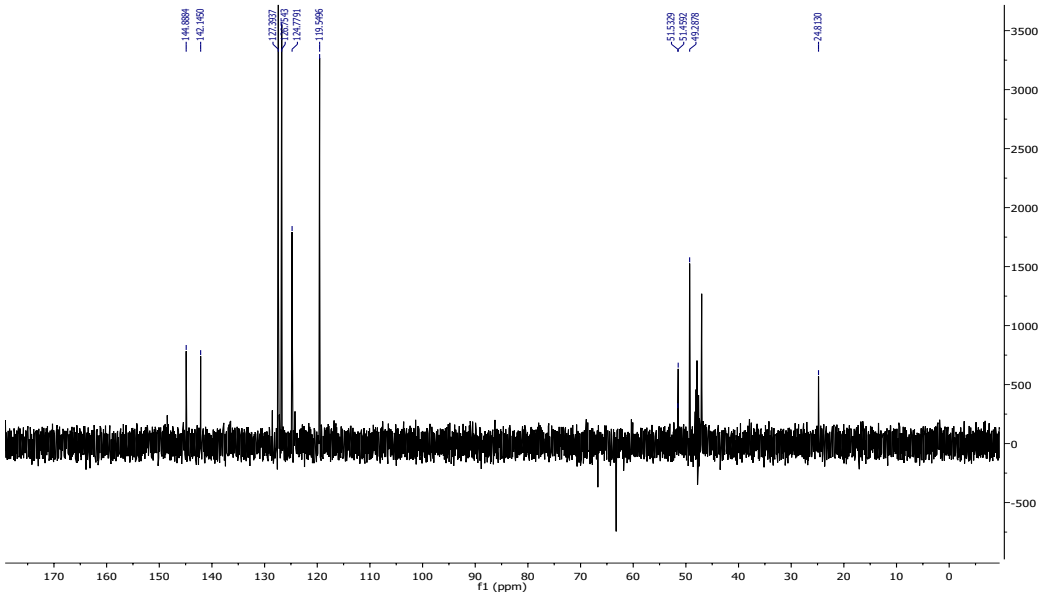


Appendix 31: COSY NMR Fmoc-dioxazole-OMe (11).

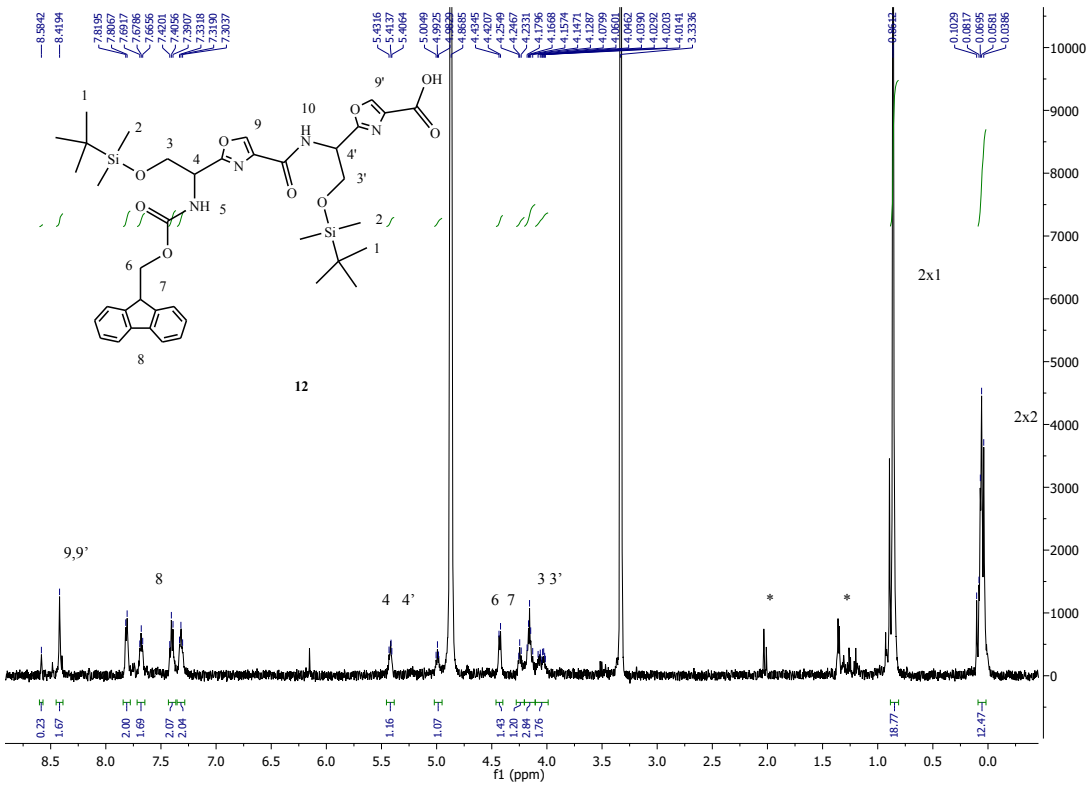
The two signals at 8.50 and 8.42 ppm represents to the two protons from the oxazole rings.



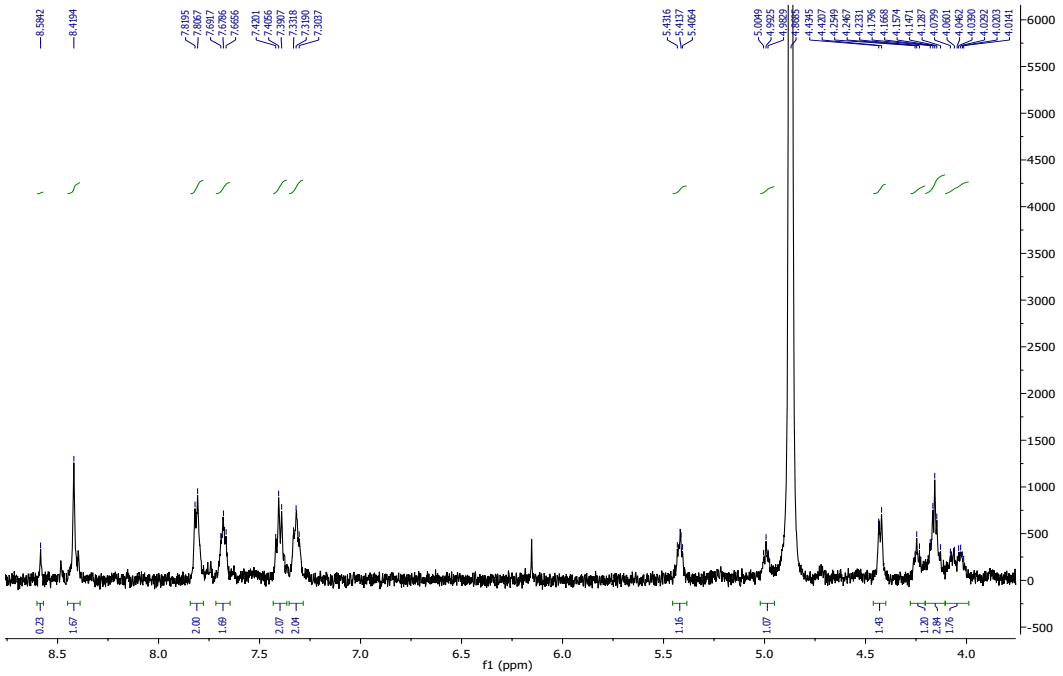
Appendix 32: ^{13}C NMR Fmoc-dioxazole-OMe (11).



Appendix 33: DEPT 135 NMR Fmoc-dioxazole-OMe (11).

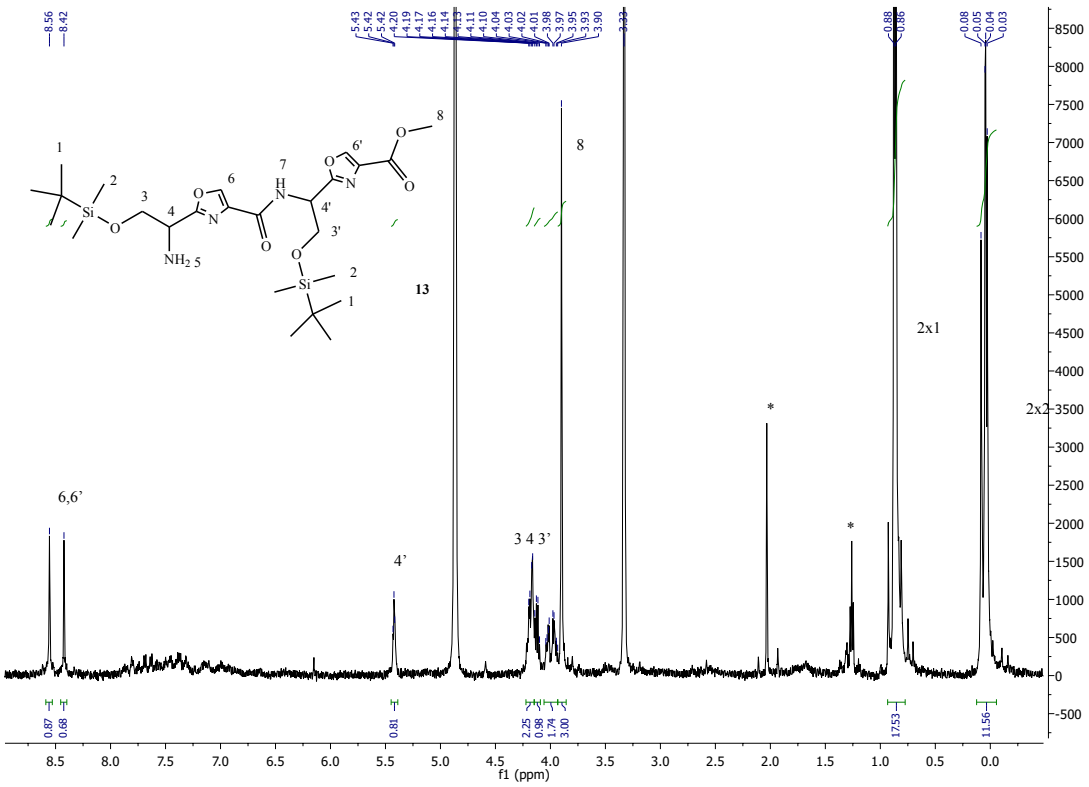


Appendix 34: ^{1}H NMR Fmoc-dioxazole-COOH (12).



- Peaks at 1.24, 2.01 and 4.10 ppm for EtOAc solvent.

The disappearance of the methyl ester signal conformed the deprotection of methyl ester group.

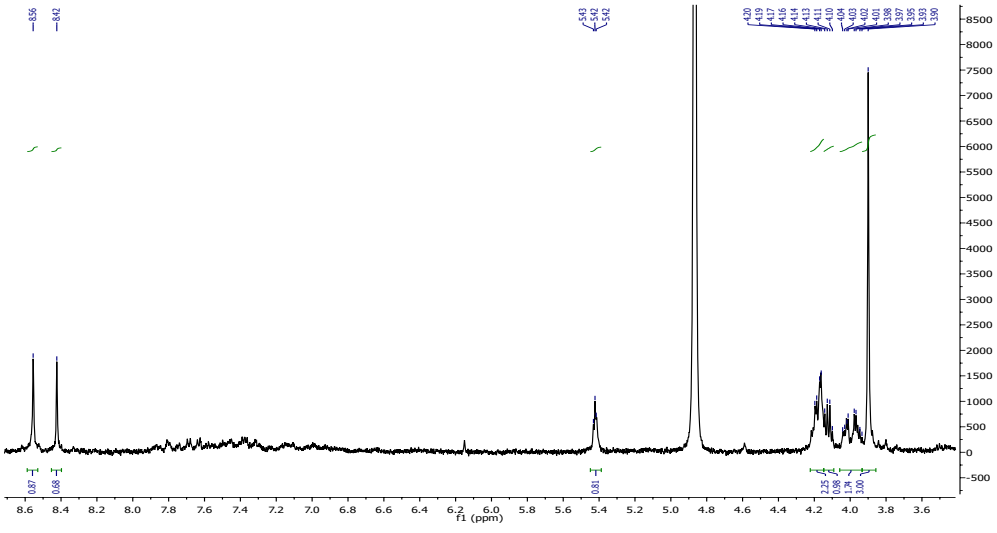


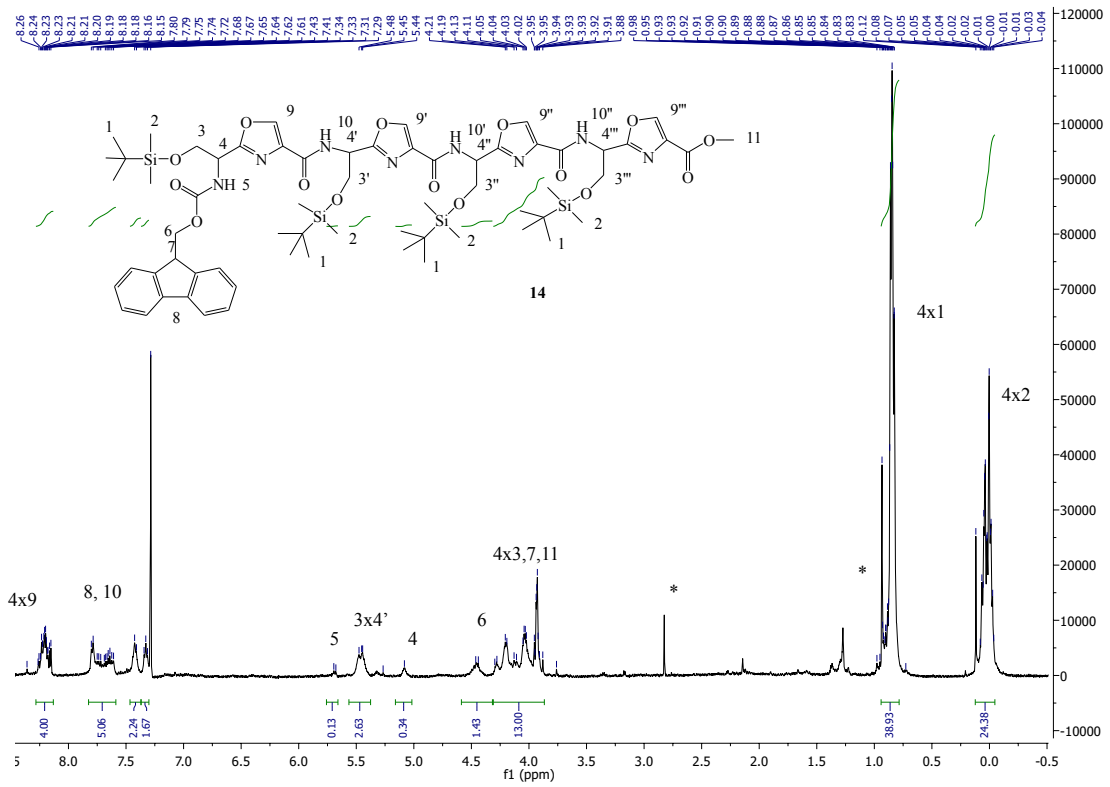
Appendix 35: ^{1}H NMR H_2N-oxazole-OMe (13).

* Peaks at 0.93 and 1.27 ppm due to hexane solvent

*Peaks at 2.11 ppm due to acetone solvent

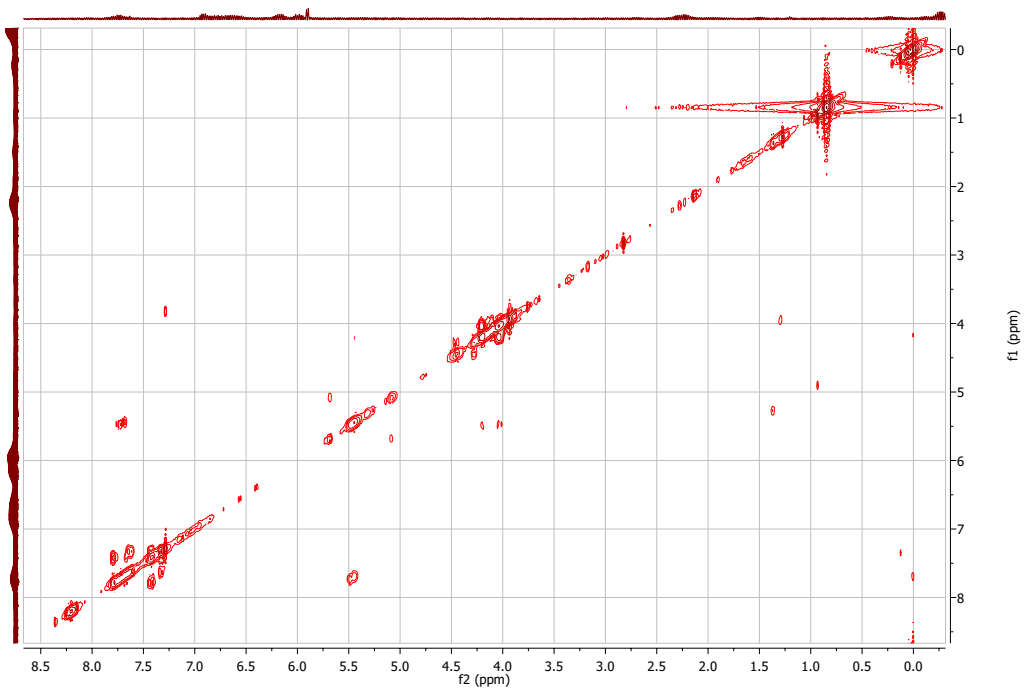
The disappearance of the Fmoc protons peaks conformed the Fmoc deprotection step.



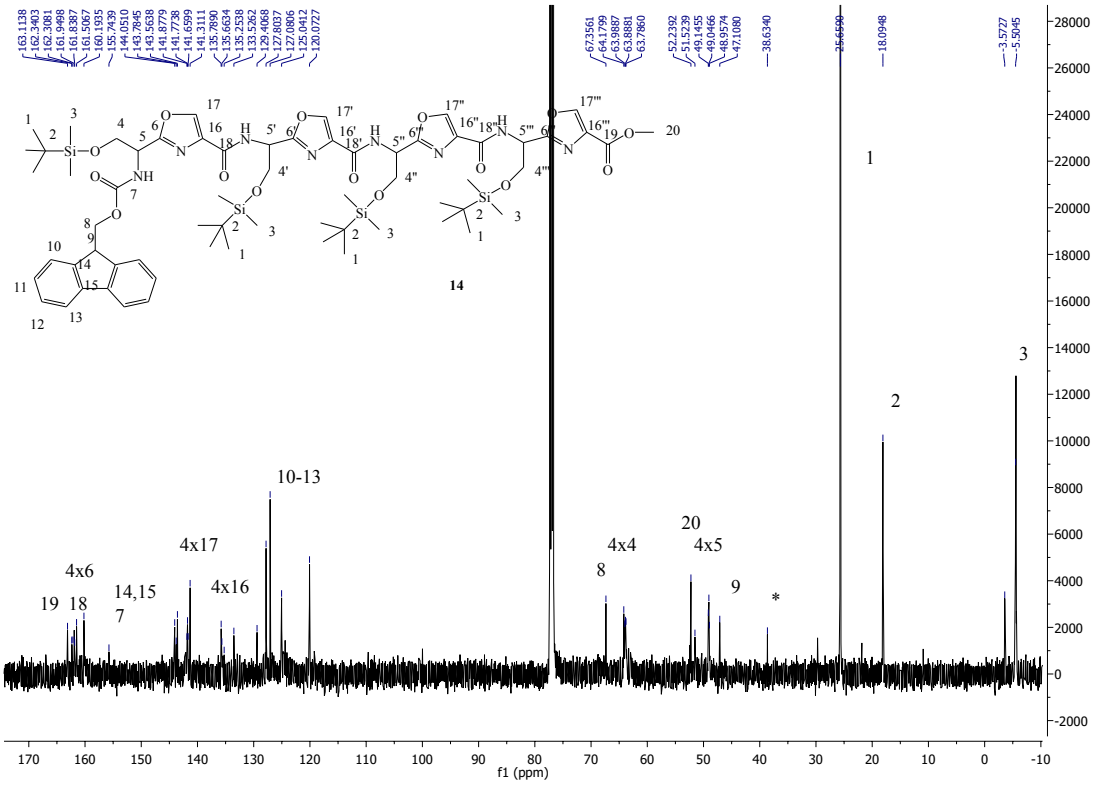


Appendix 36: ^{1}H NMR Fmoc-tetraoxazole-OMe (14).

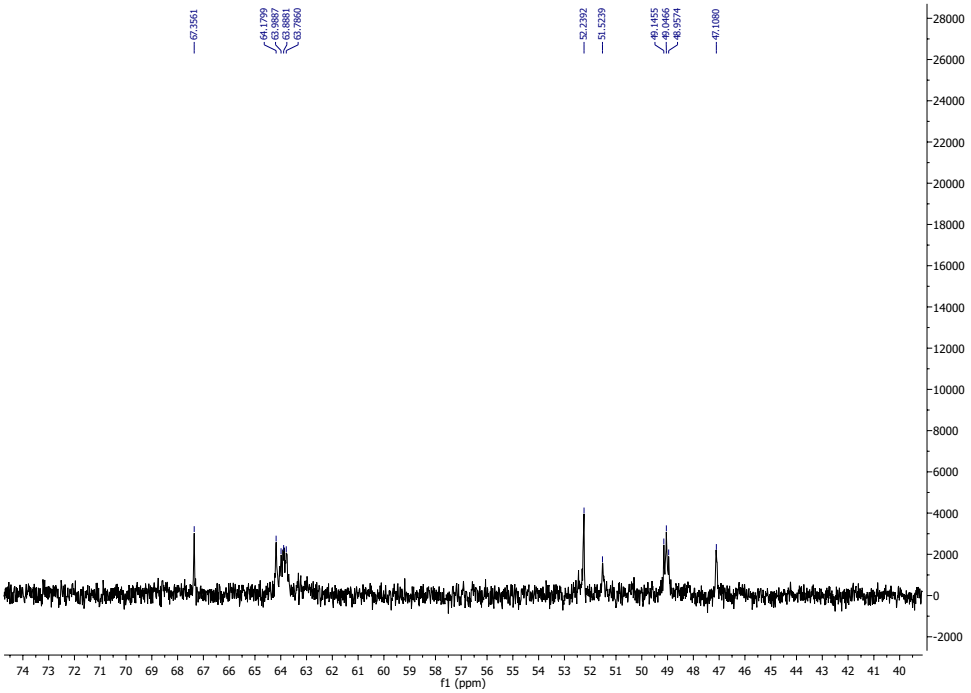
- Peaks at 0.88 and 1.26 ppm due to hexane
- Peak at 2.8 due to urea by-product

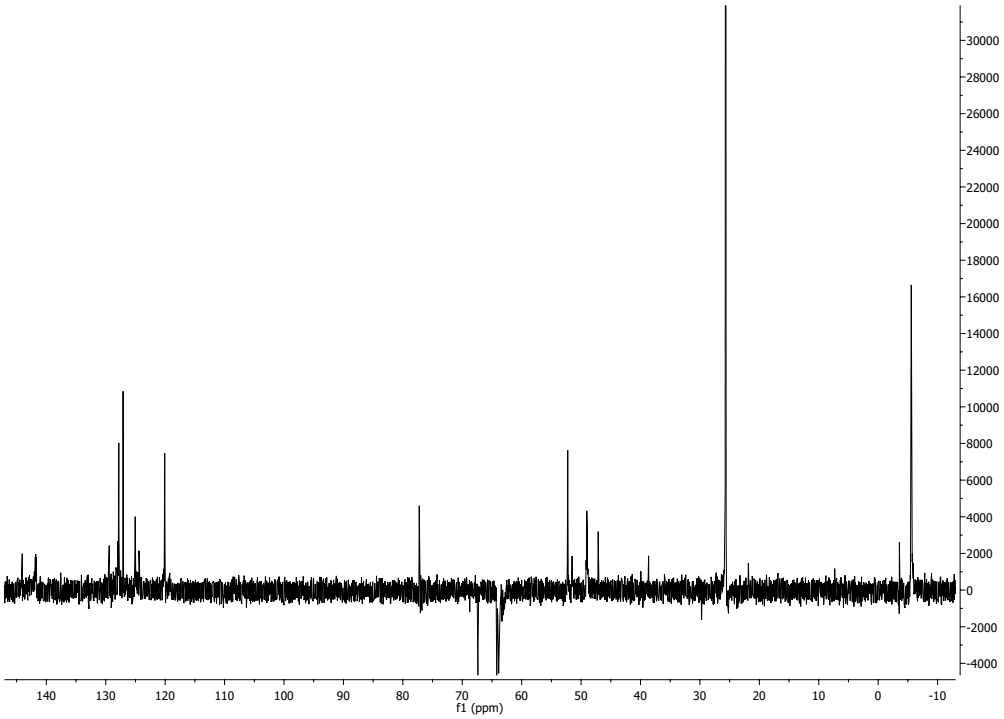


Appendix 37: COSY NMR Fmoc-tetraoxazole-OMe (14).

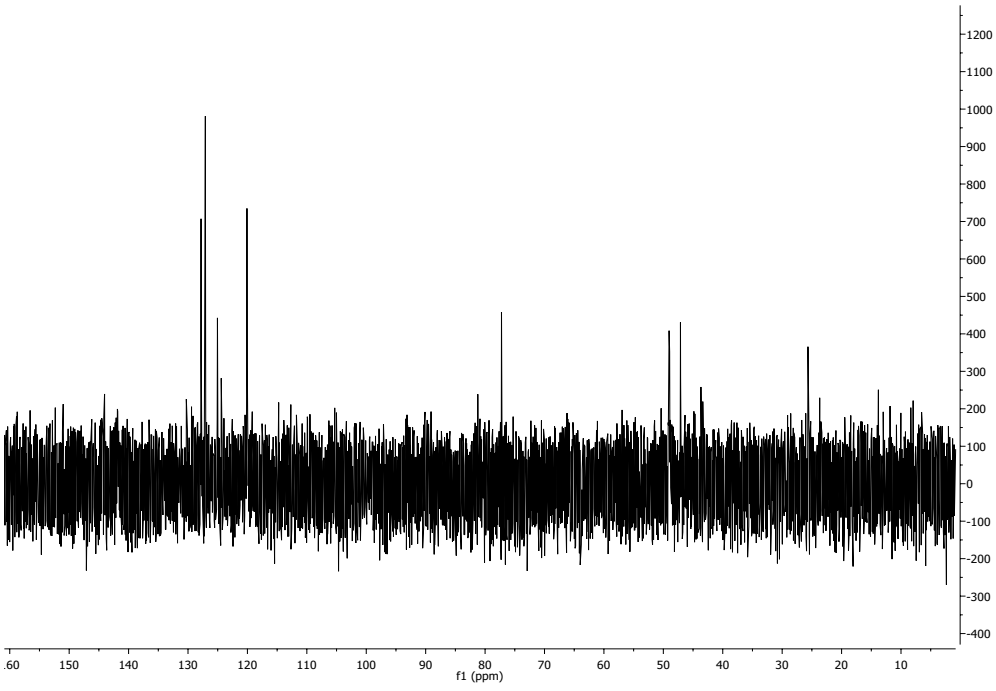


Appendix 38: ^{13}C NMR Fmoc-tetraoxazole-OMe (14).

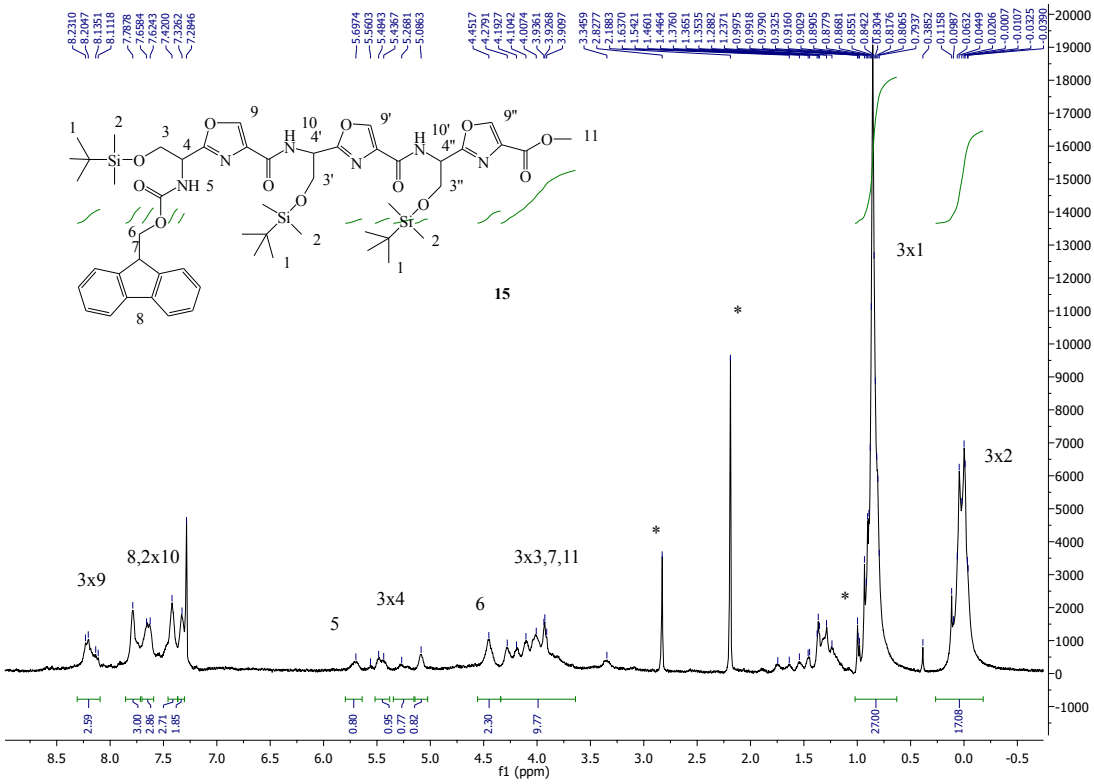




Appendix 39: DEPT 135 NMR Fmoc-tetraoxazole-OMe.

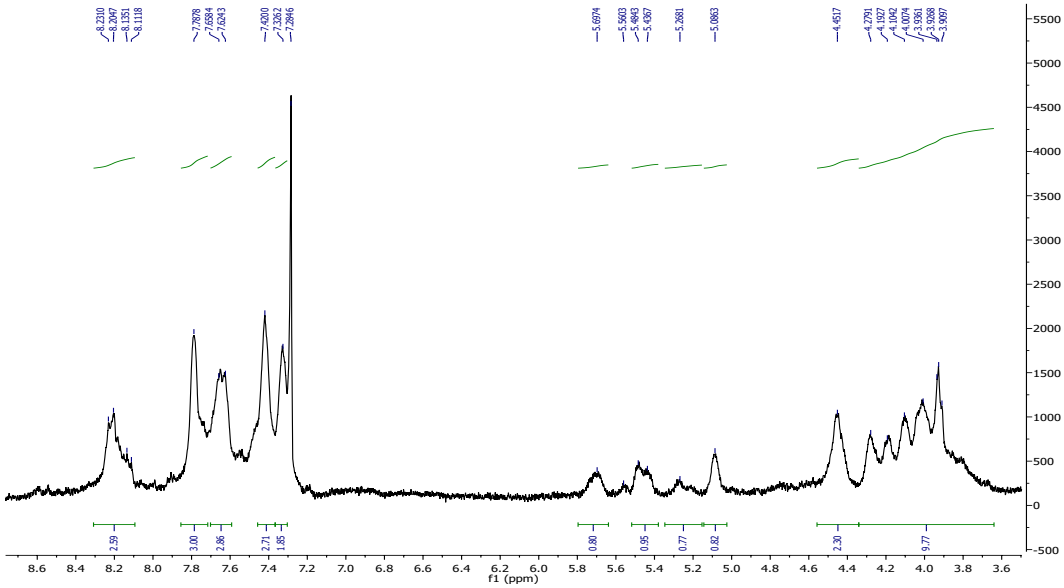


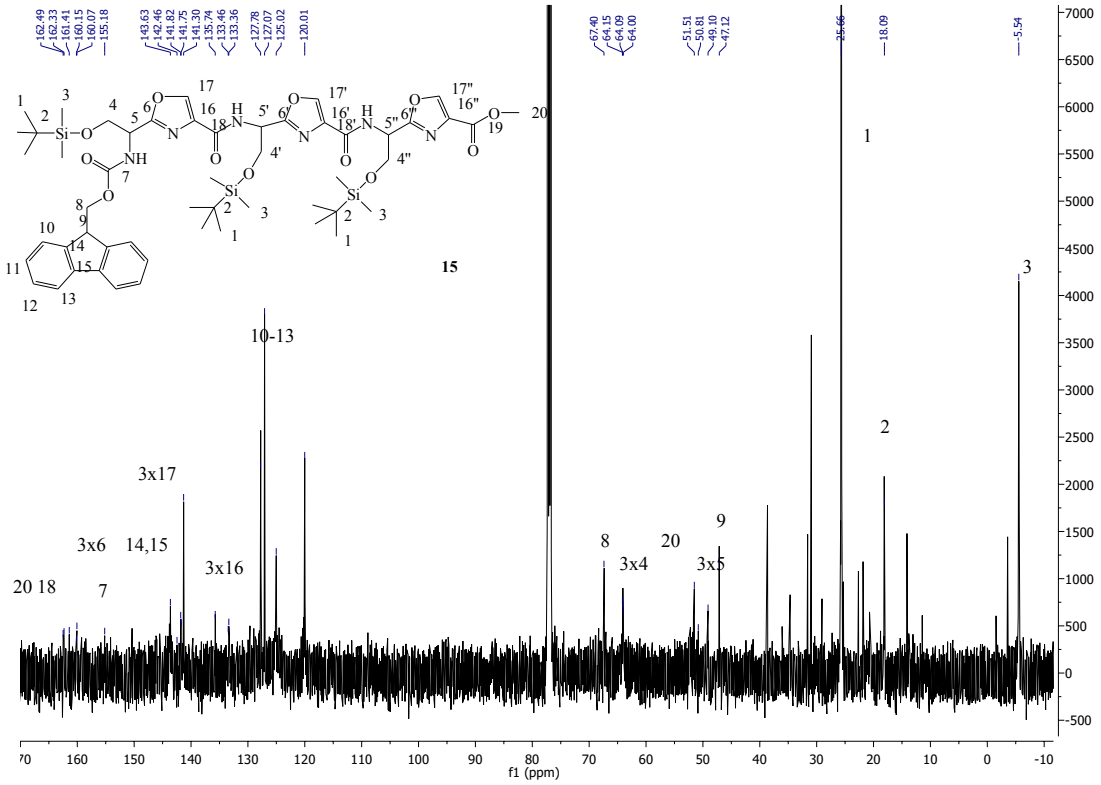
Appendix 40: DEPT 90 NMR Fmoc-tetraoxazole-OMe (14).



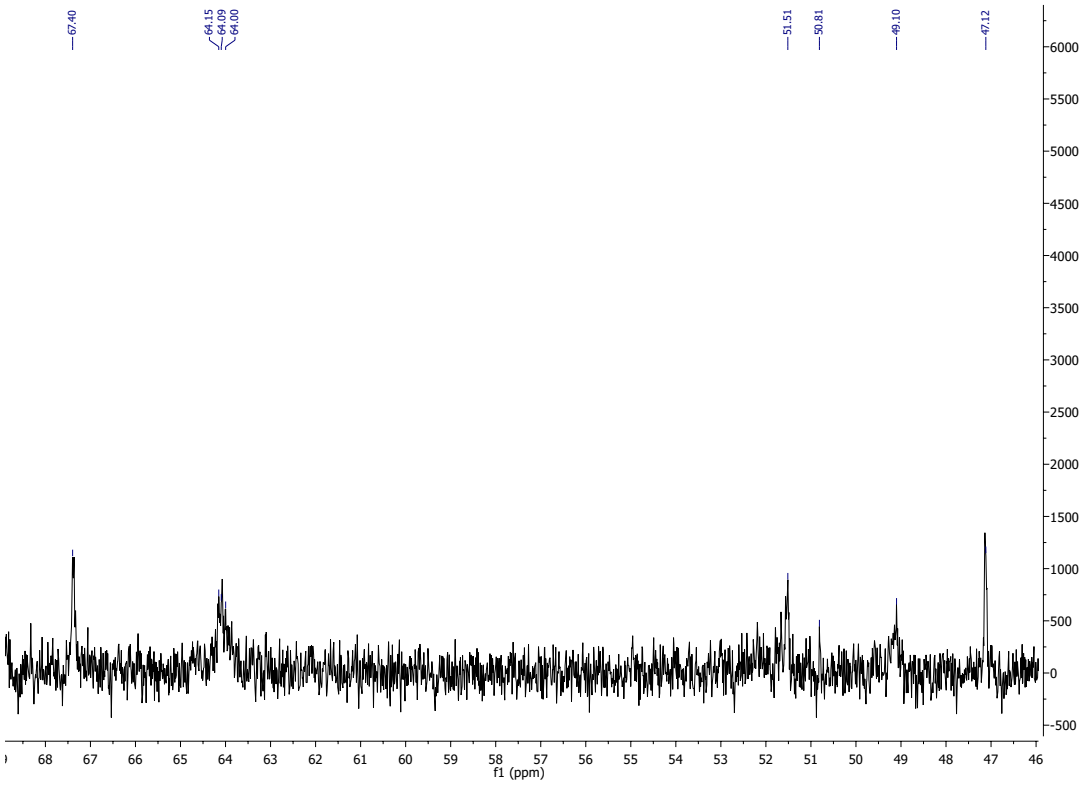
Appendix 41: ^{1}H NMR Fmoc-trioxazole-OMe (15).

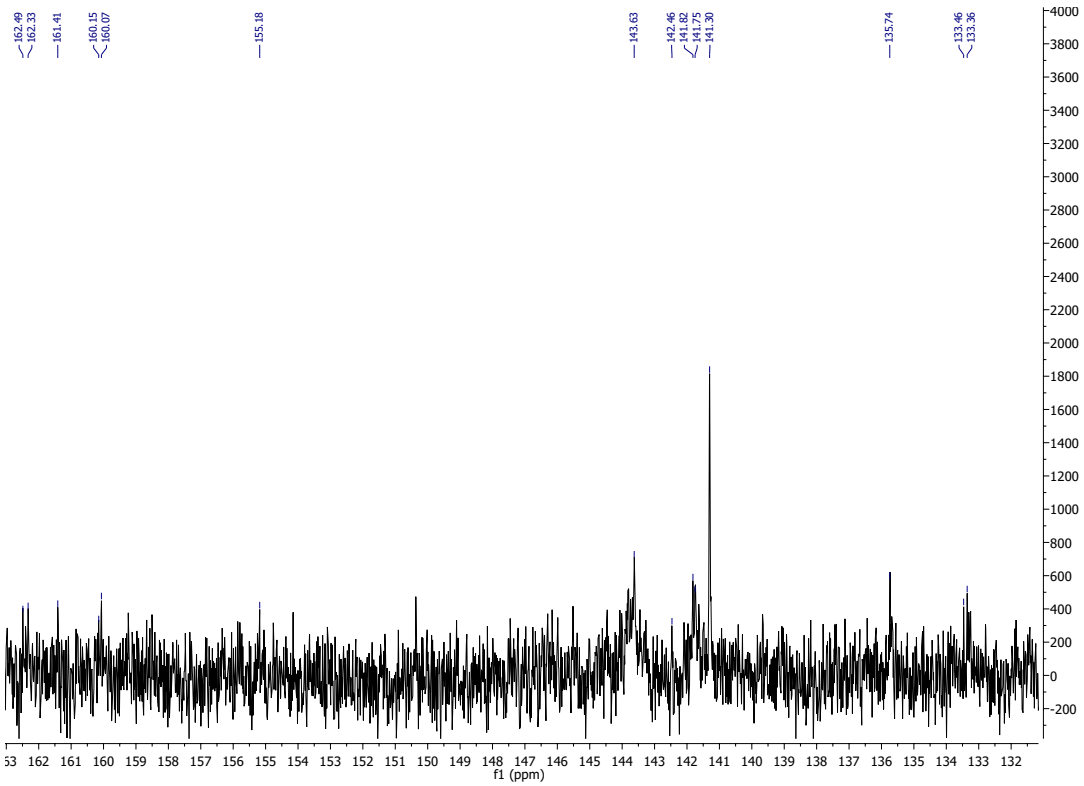
- Peaks at 0.88 and 1.26 ppm due to hexane
- Peak at 2.1 ppm due to acetone
- Peak at 2.8 due to urea by-product

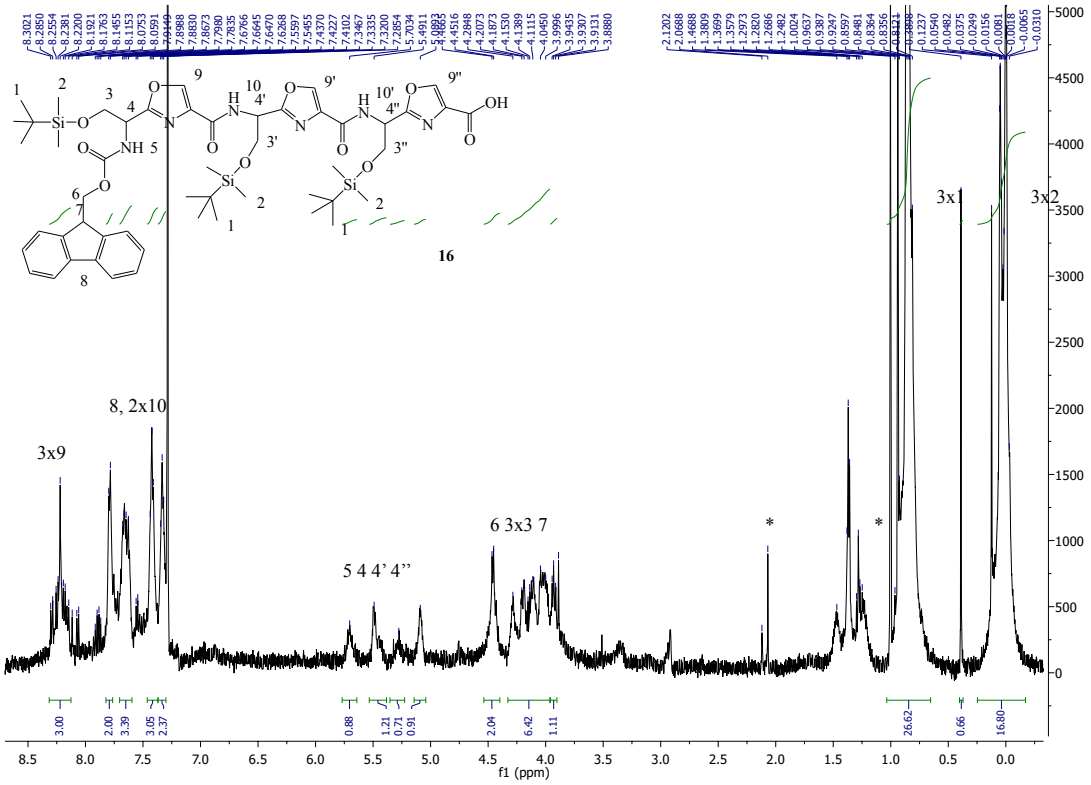




Appendix 42: ^{13}C NMR Fmoc-trioxazole-OMe (15).

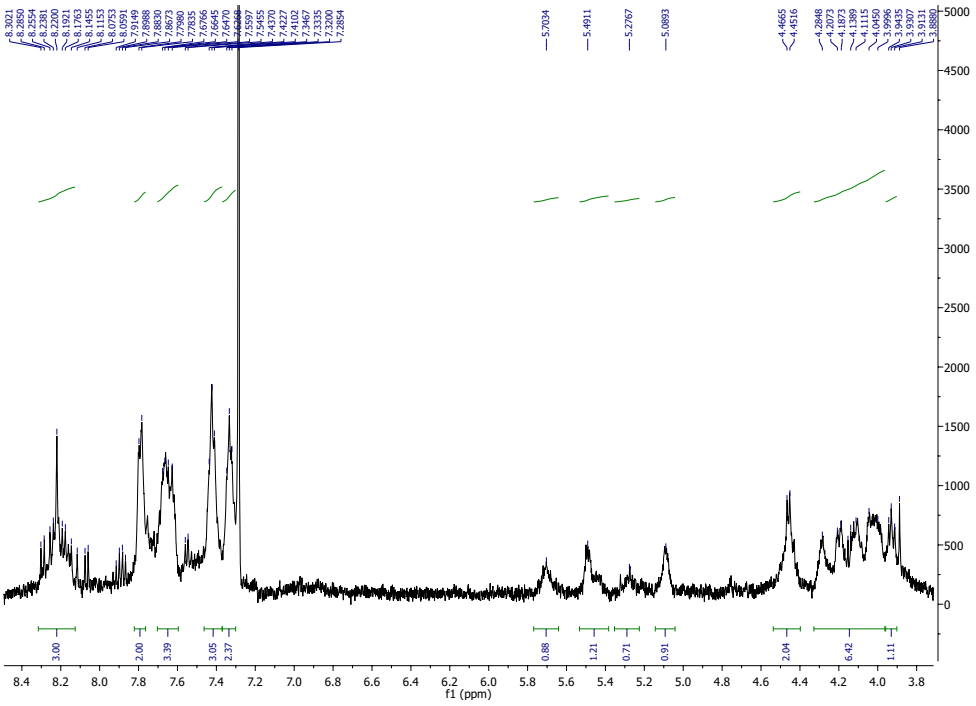

67.40
64.15
64.09
64.00
51.51
50.81
49.10
47.12
f1 (ppm)

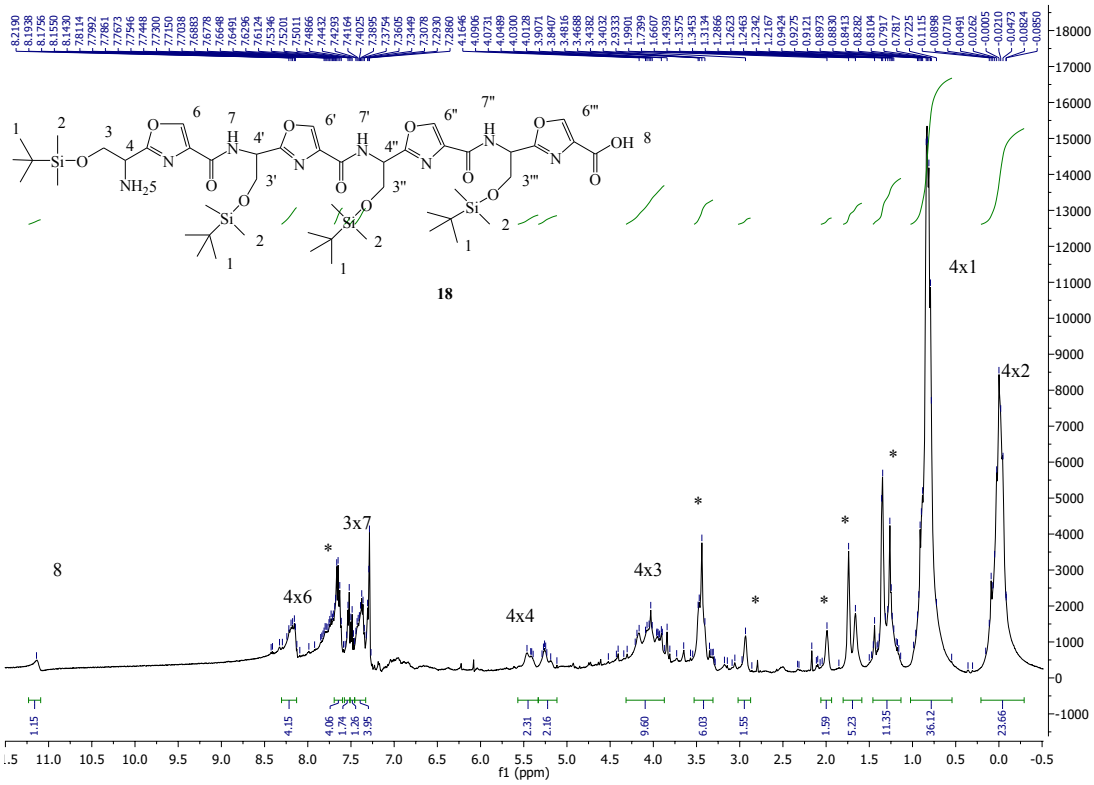

162.49
162.33
161.41
160.15
160.07
155.18
143.63
142.46
141.82
141.75
141.30
135.74
133.46
133.36
f1 (ppm)



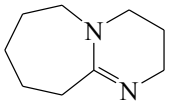
Appendix 43: ^{1}H NMR Fmoc-trioxazole-COOH (16).

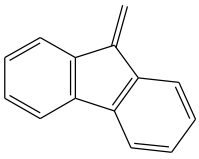
* Peaks due to EtOAc solvent

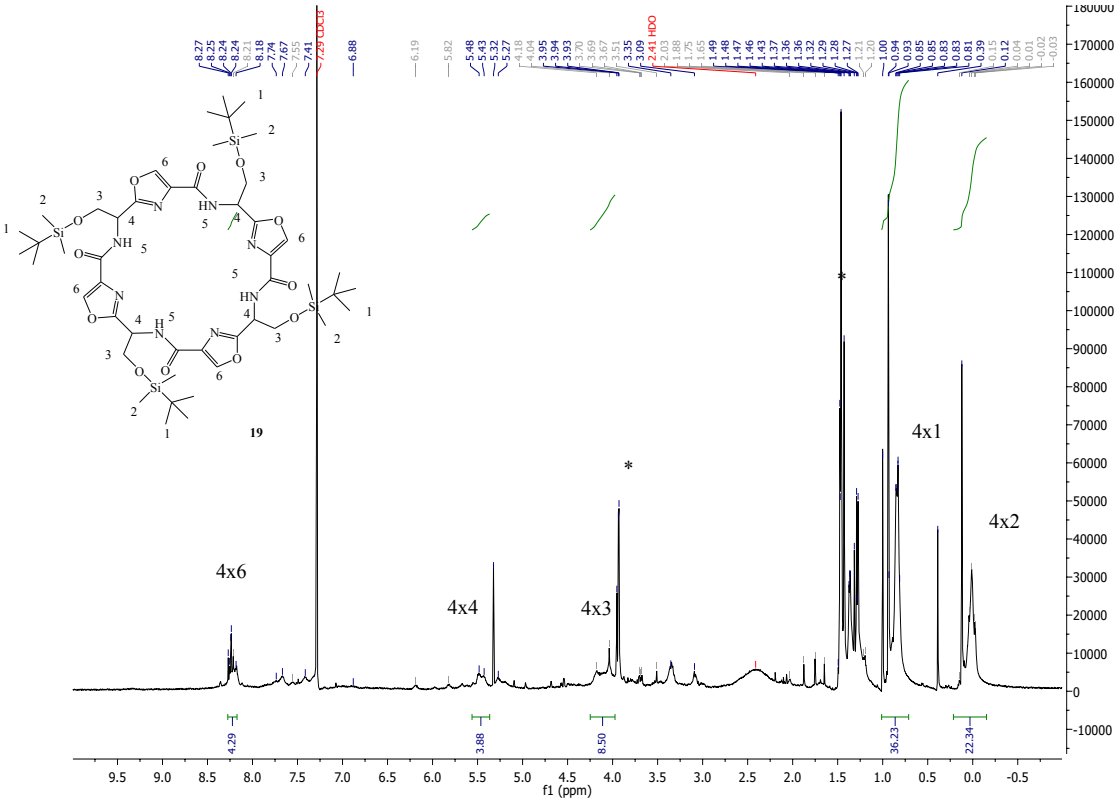




Appendix 44: ^{1}H NMR H_2N-tetraoxazole-COOH (18).

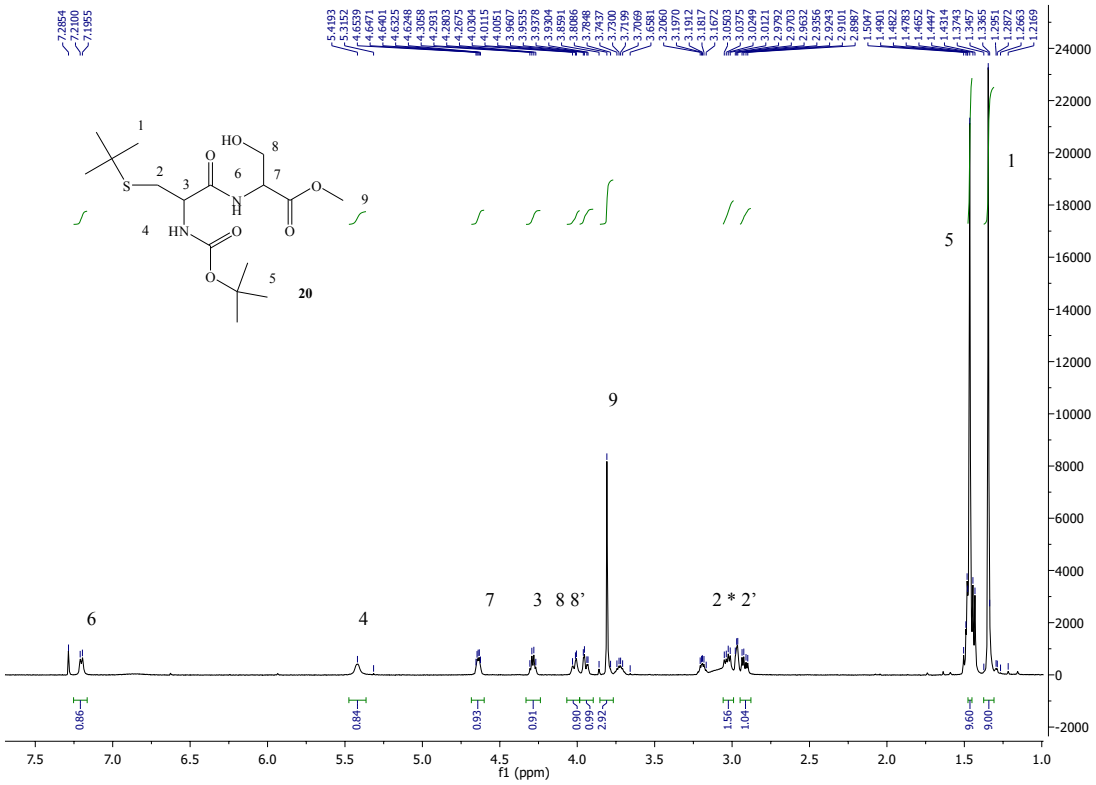
- Peaks at 4.32, 3.53, 2.95, 1.70, 1.46 ppm due to DBU base 
- Peaks at 7.69 – 7.60 (m, 4H), 7.39 (m, 4H) due to by-product from Fmoc cleavge





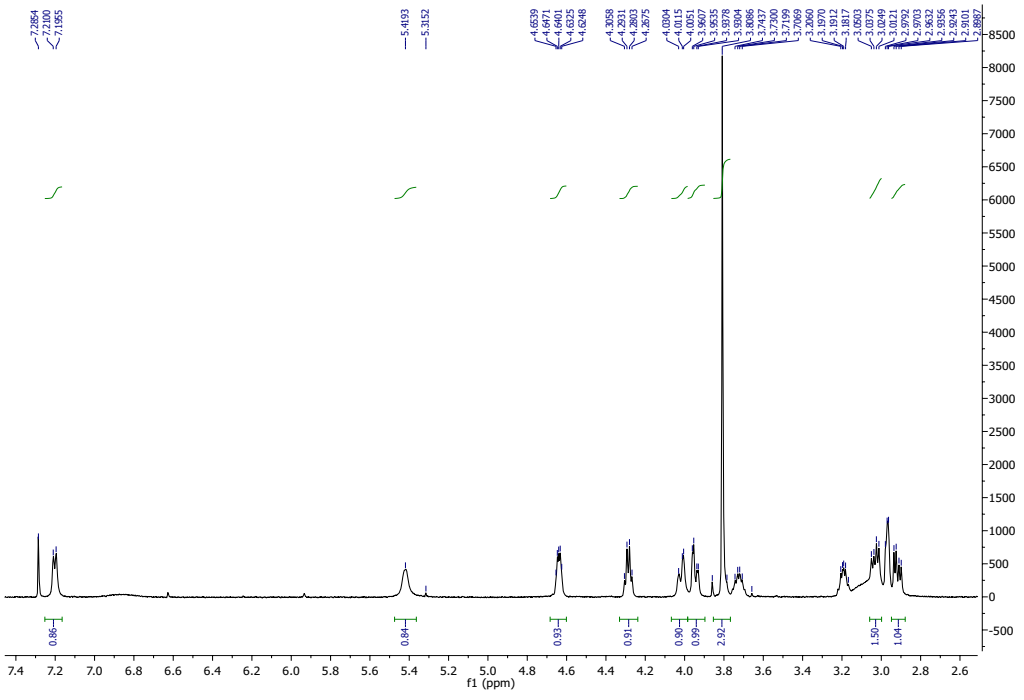
Appendix 45: ^{1}H NMR tetraoxazole macrocyclic peptide with TBS groups (19).

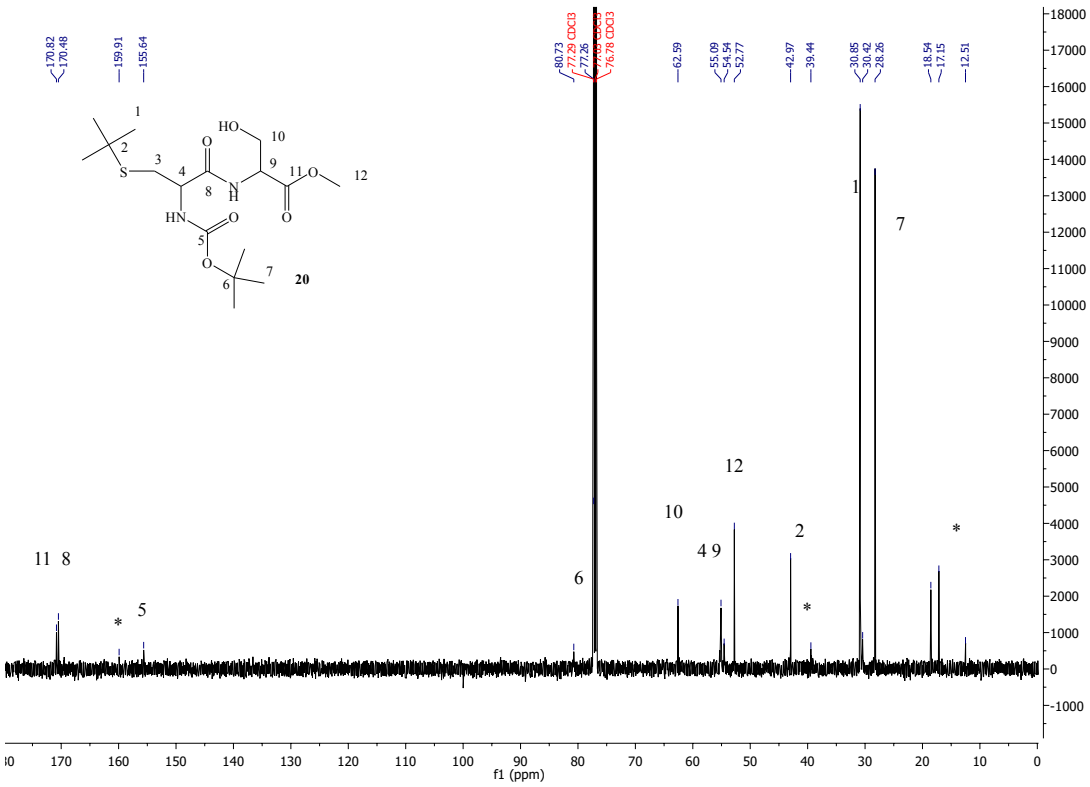
* Peak at 1.28 and 3.93 ppm are unknown



Appendix 46: ^{1}H NMR Boc-Cys(*t*-Bu)-OH-Ser-OMe (20).

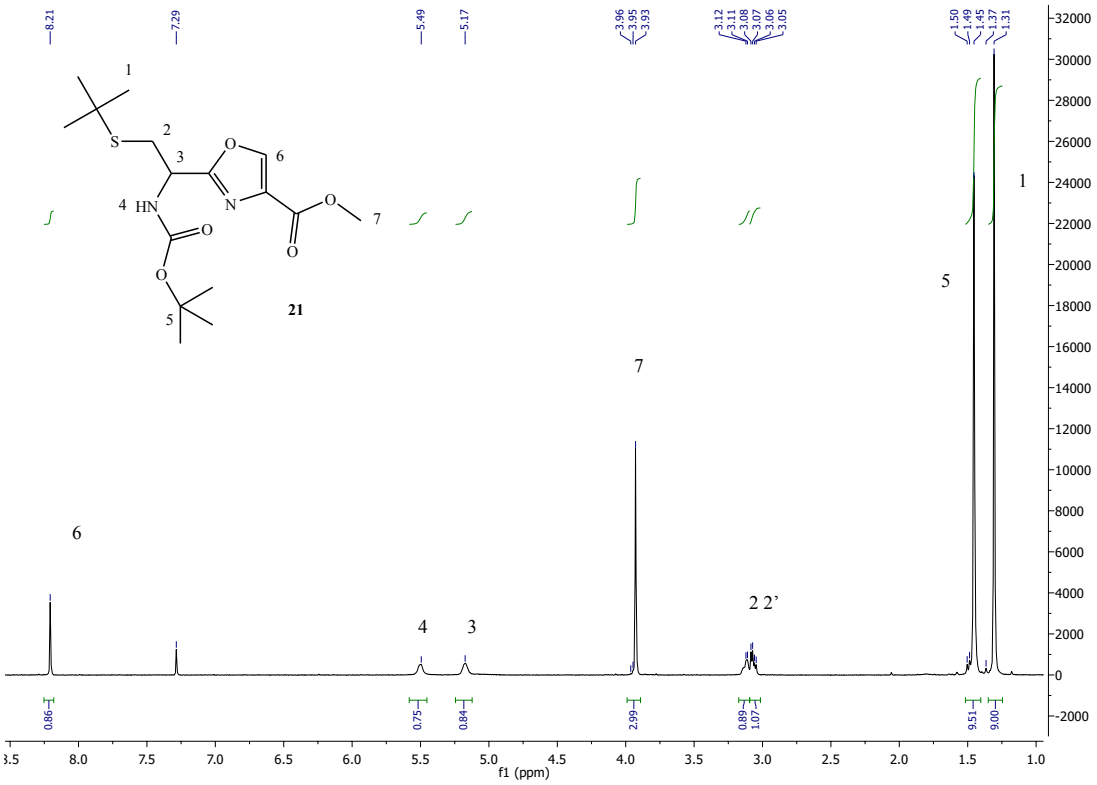
* Peak at 2.8 ppm due to urea by-product



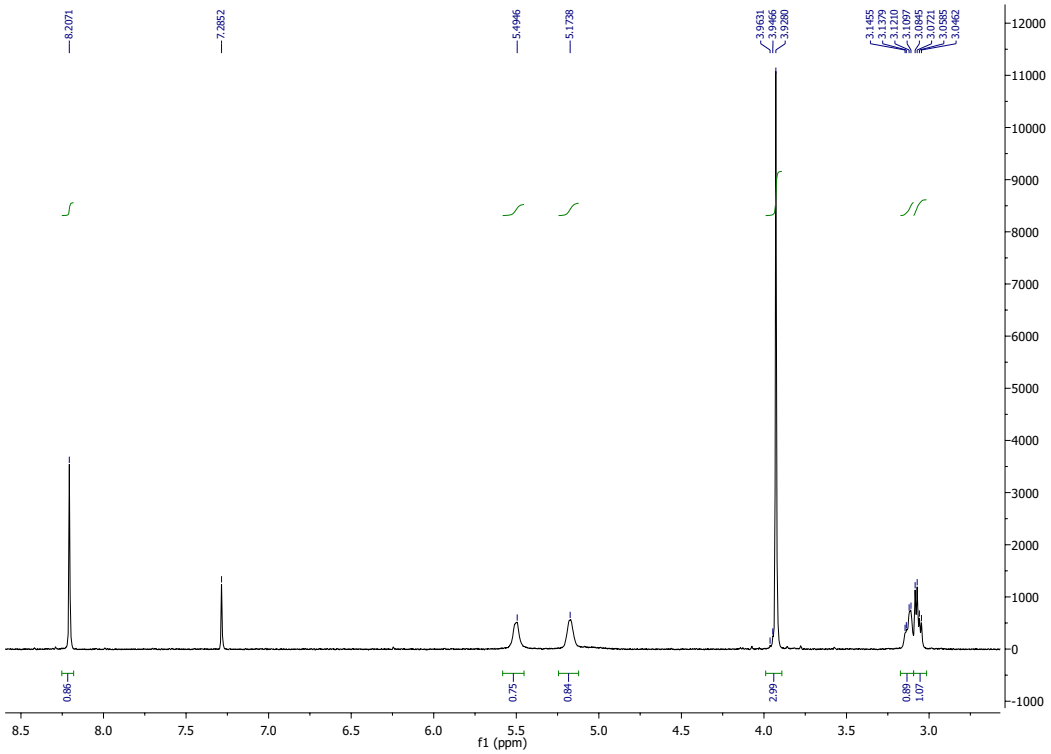


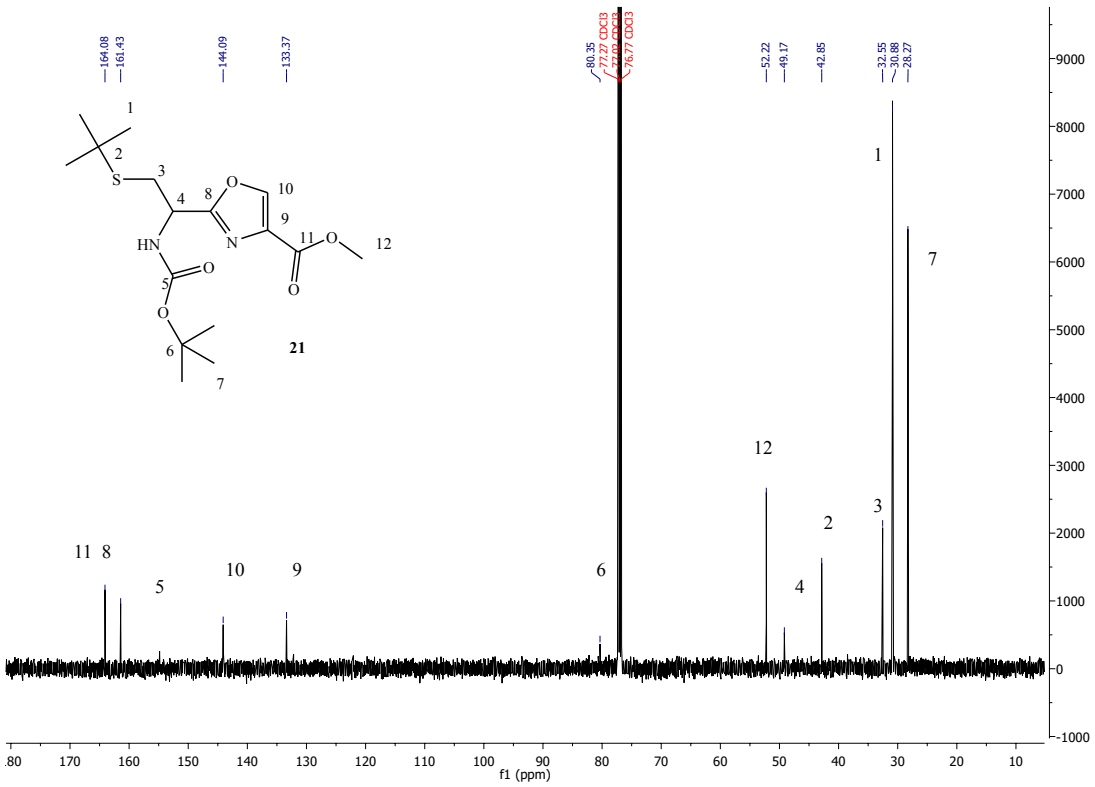
Appendix 47: ^{13}C NMR Boc-Cys(*t*-Bu)-OH-Ser-OMe (20).

* Peaks at 159.91, 39.44 from urea by-product.

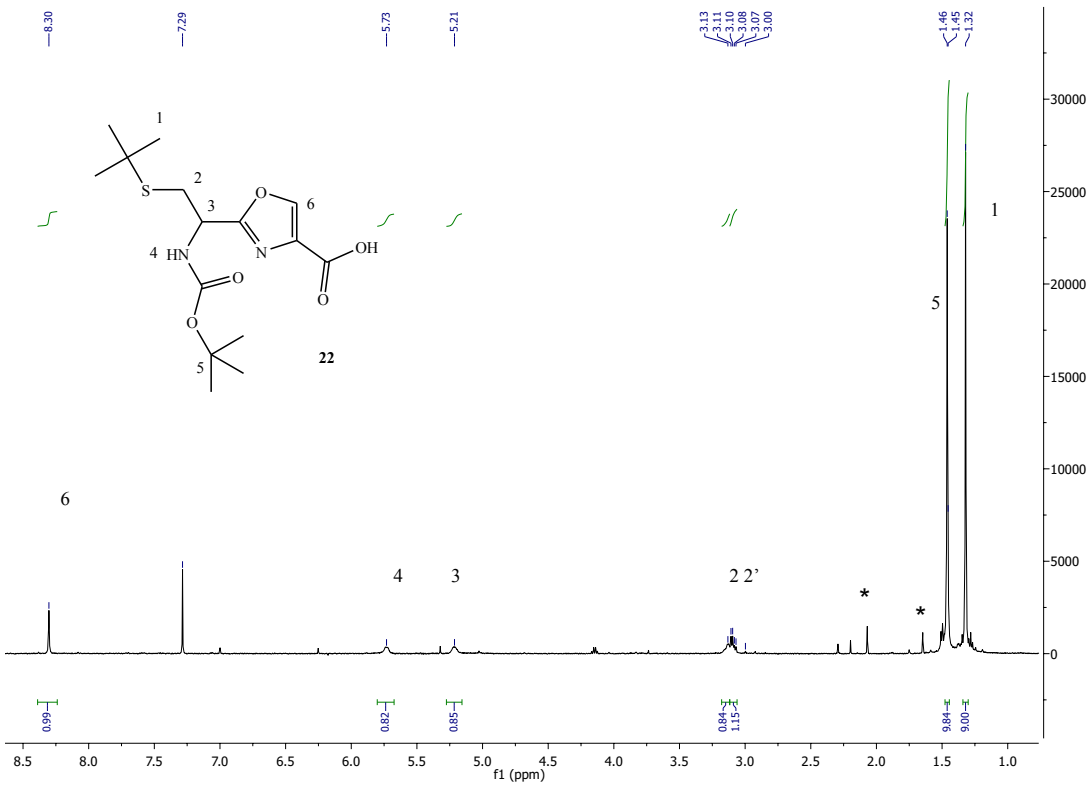


Appendix 48: ^{1}H NMR Boc-oxazole-OMe (21).

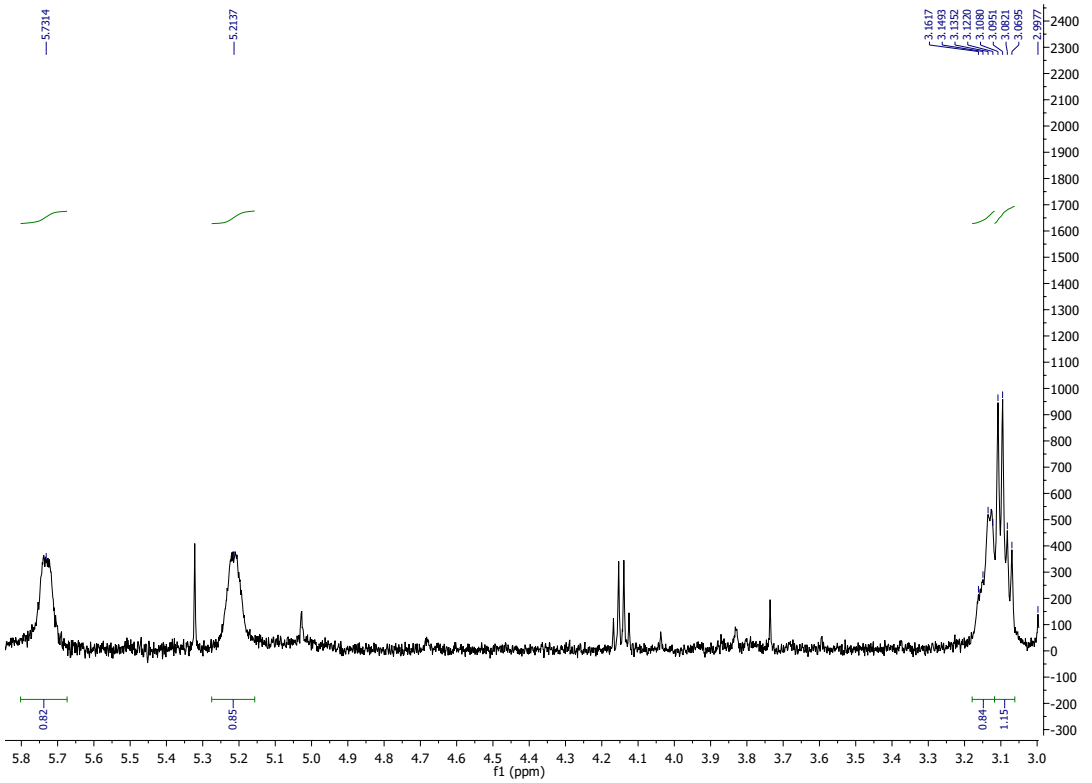


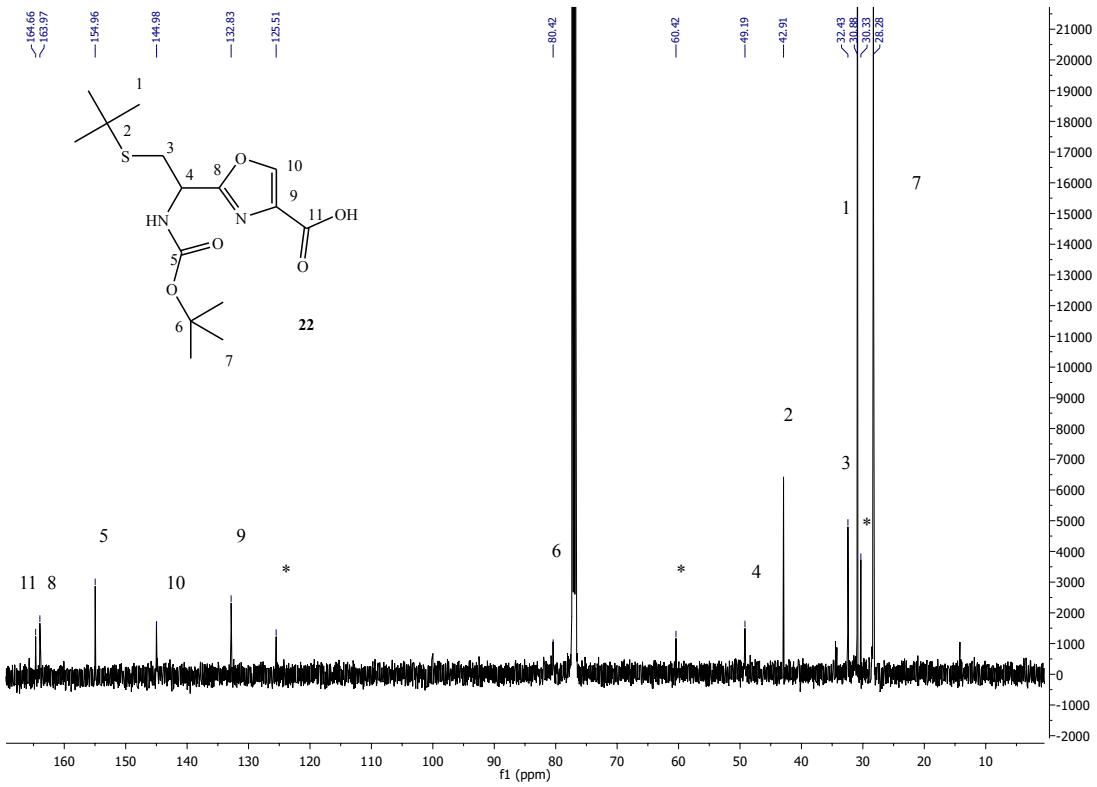


Appendix 49: ^{13}C NMR Boc-oxazole-OMe (21).



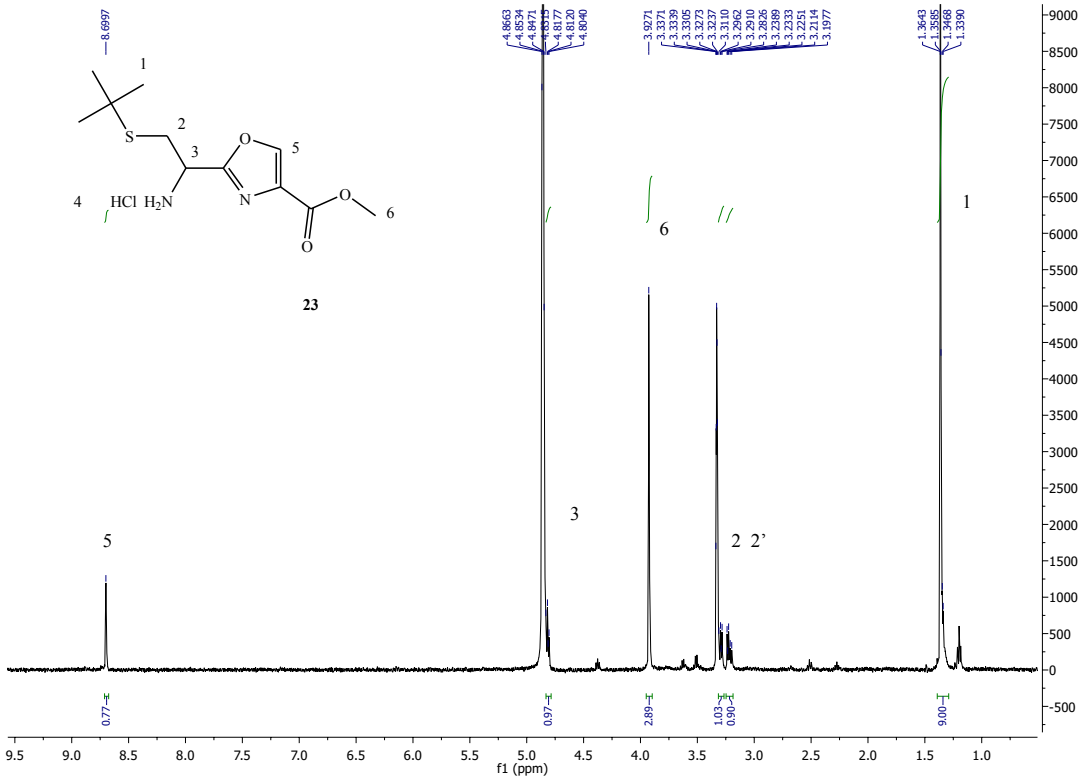
Appendix 50: ^{1}H NMR Boc-oxazole-COOH (22).



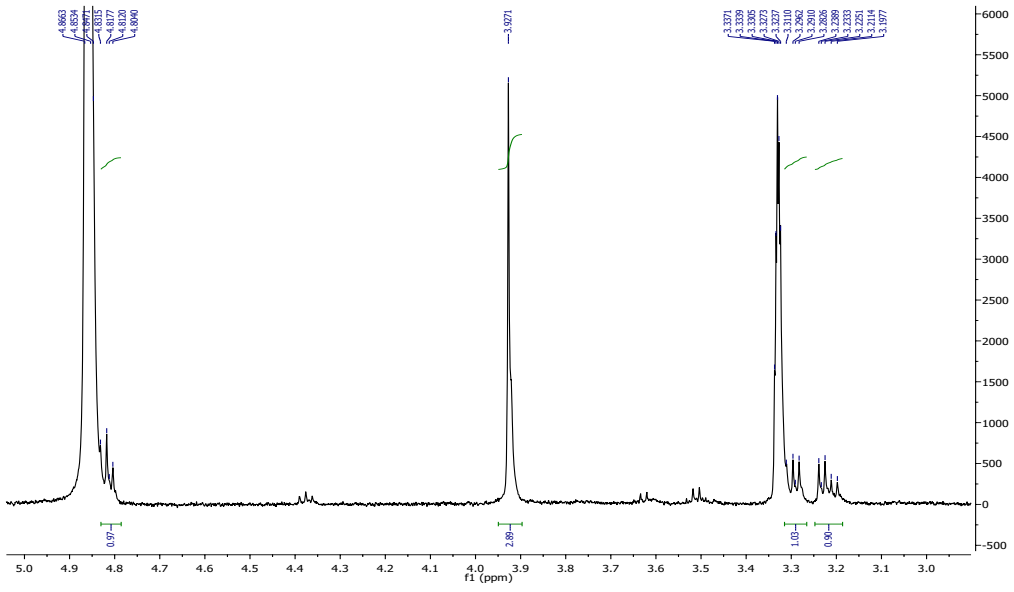


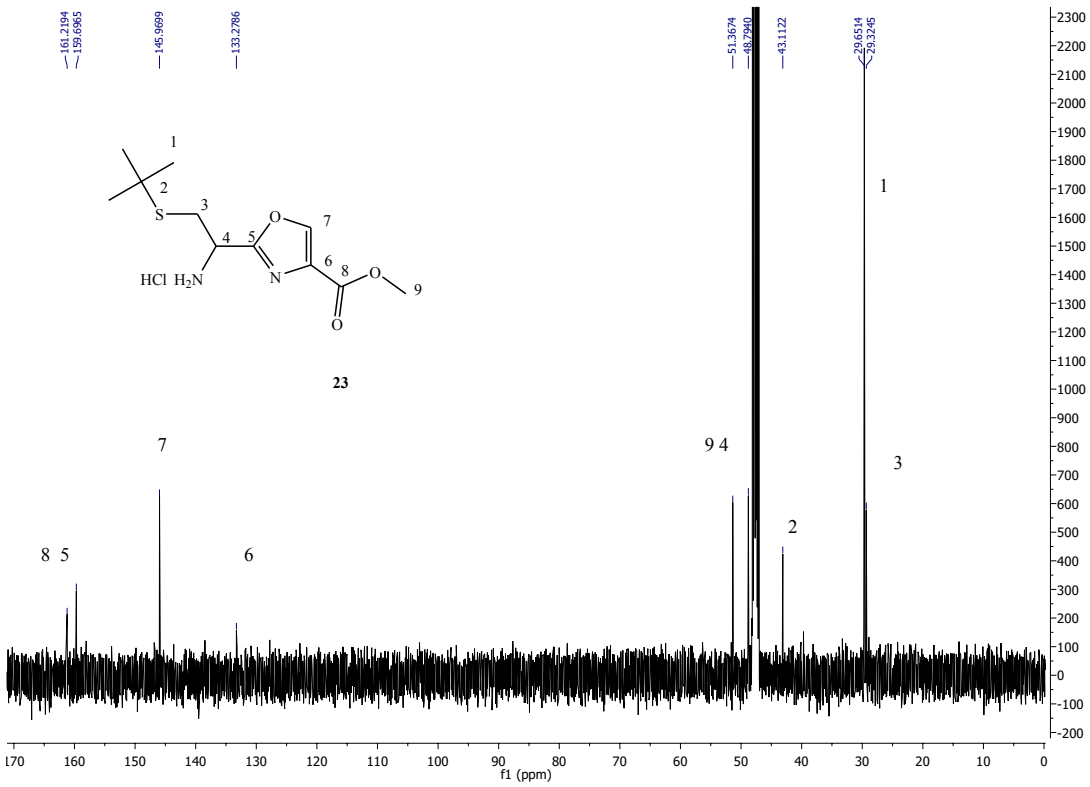
Appendix 51: ^{13}C NMR Boc-oxazole-COOH (22).

- Peaks due to EtOAc and acetone solvents

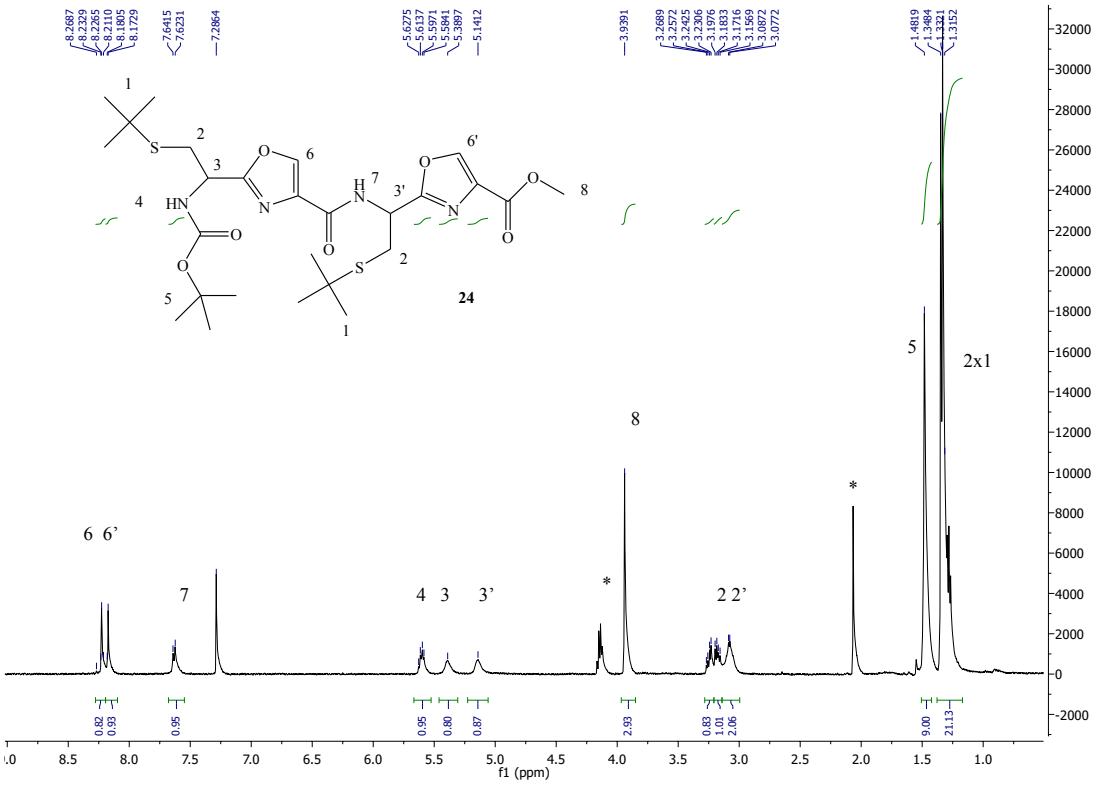


Appendix 52: ^{1}H NMR HCl.H_2N-oxazole-OMe (23).



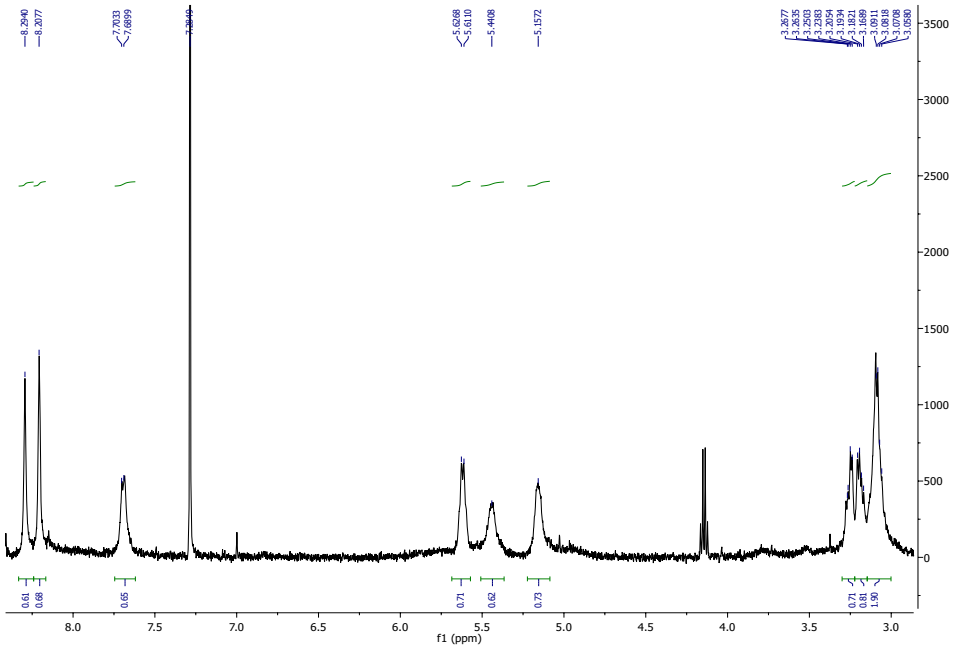


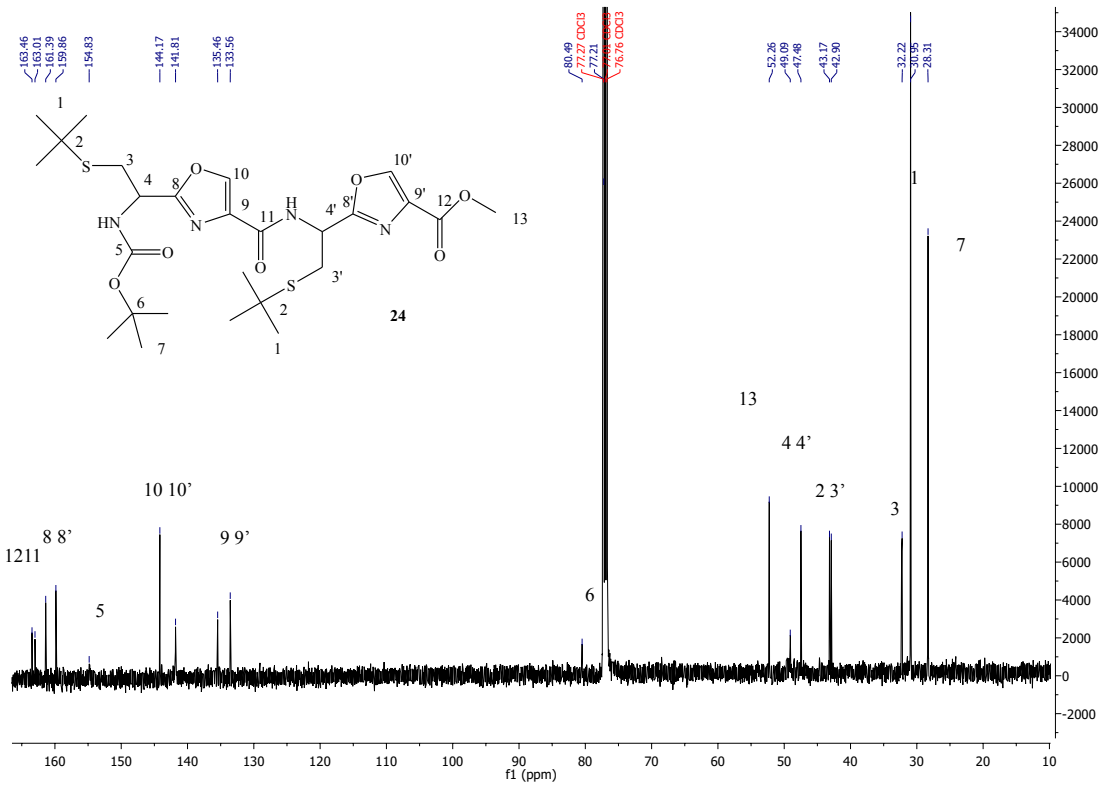
Appendix 53: ^{13}C NMR HCl.H_2N-oxazole-OMe (23).



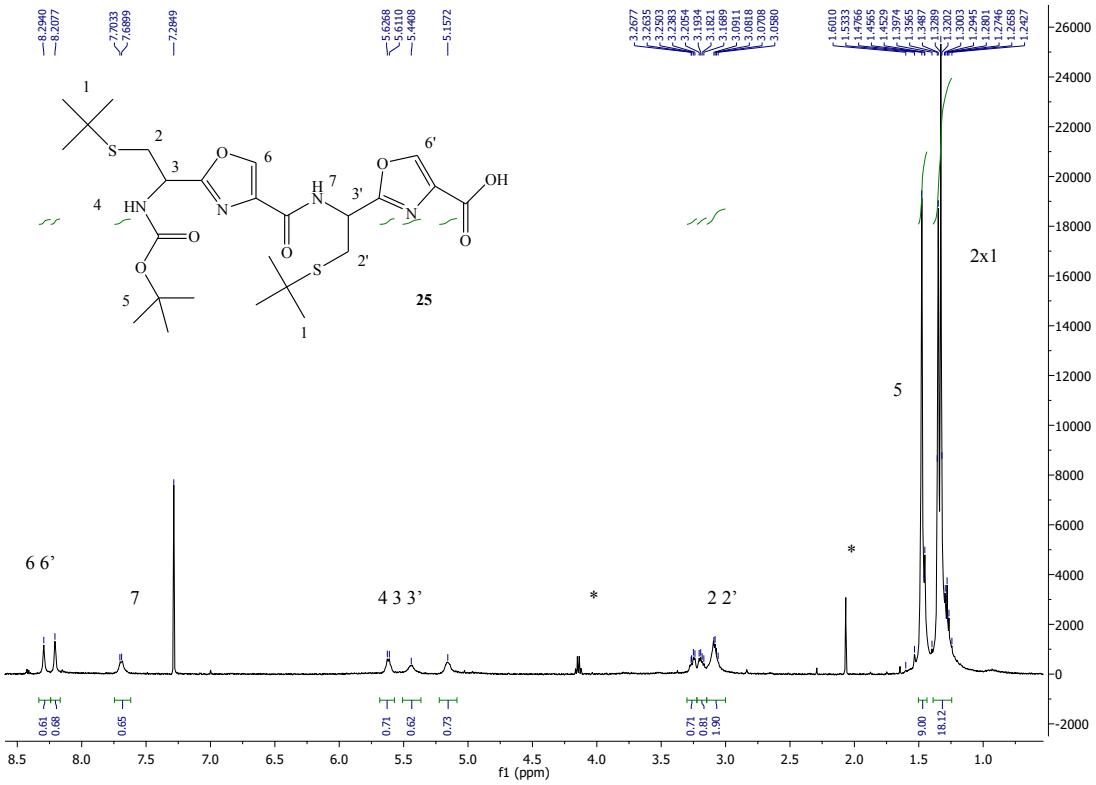
Appendix 54: ^{1}H NMR Boc-dioxazole-OMe (24).

* Peaks due to EtOAc solvent, *t*-Bu as a multiplet and the integration is higher than 18H because it is overlap with peak from EtOAc.



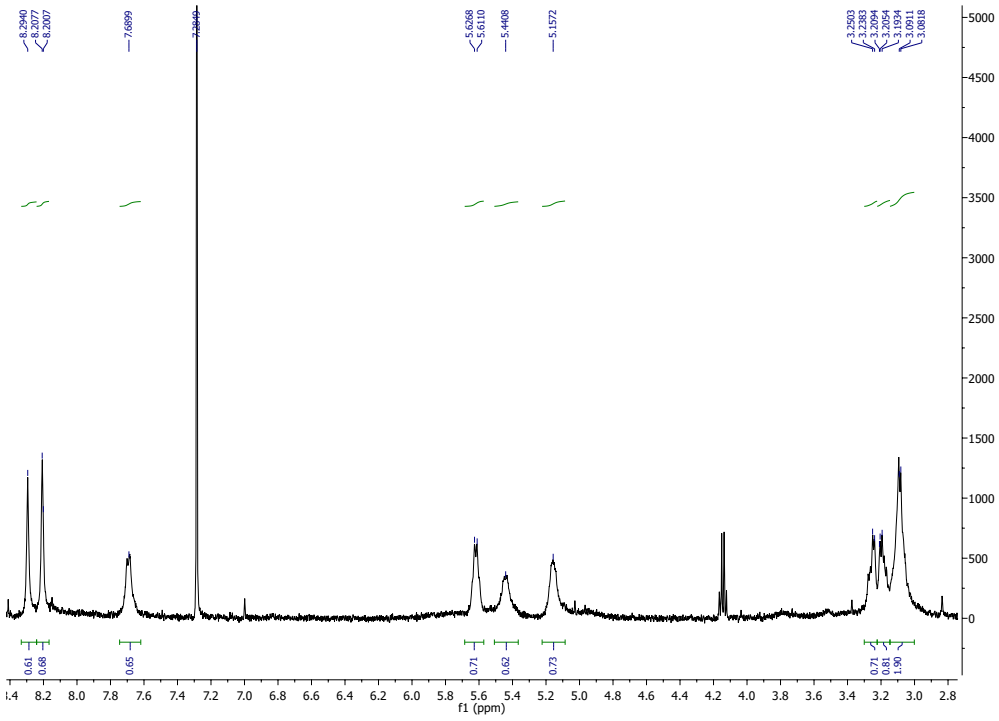


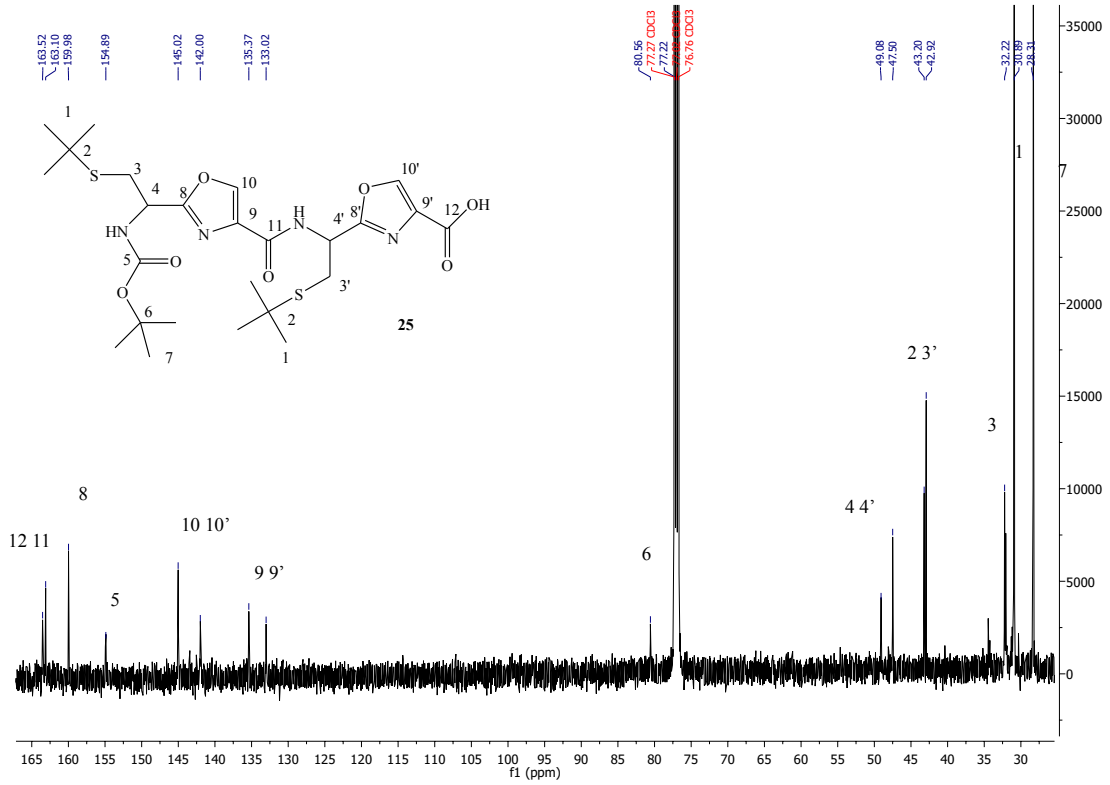
Appendix 55: ^{13}C NMR Boc-dioxazole-OMe (24).



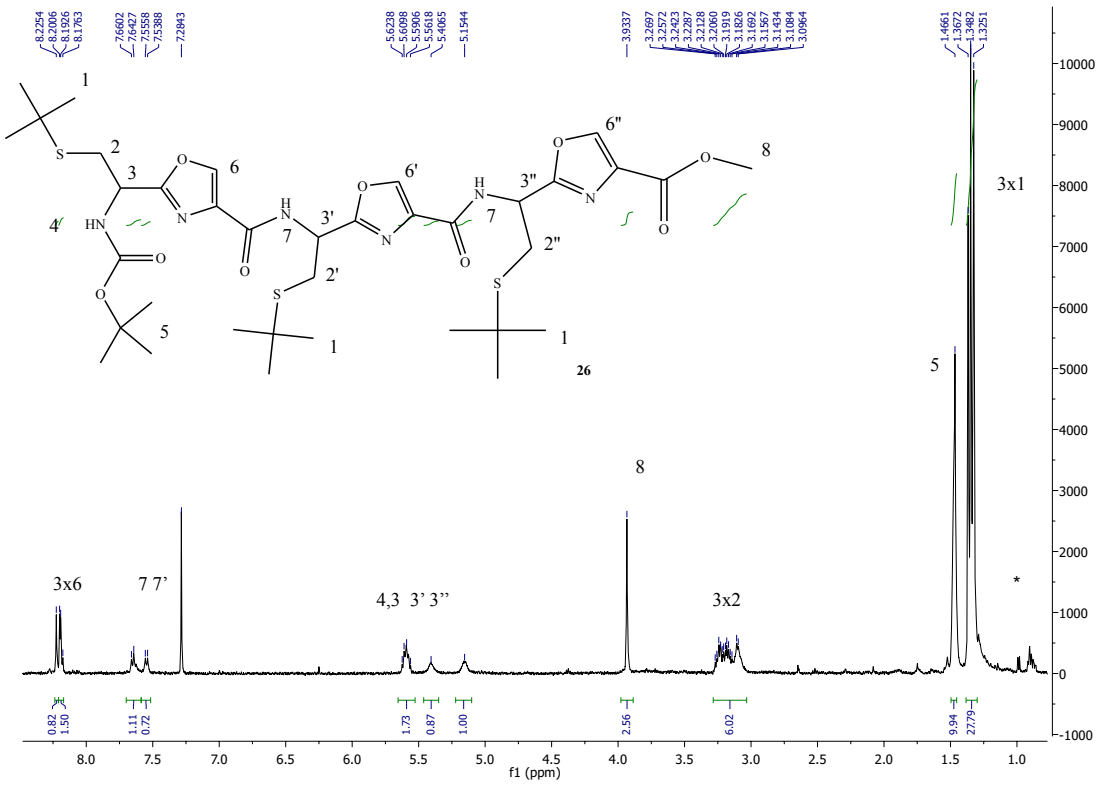
Appendix 56: ^{1}H NMR Boc-dioxazole-COOH (25).

- Peaks due to EtOAc solvent

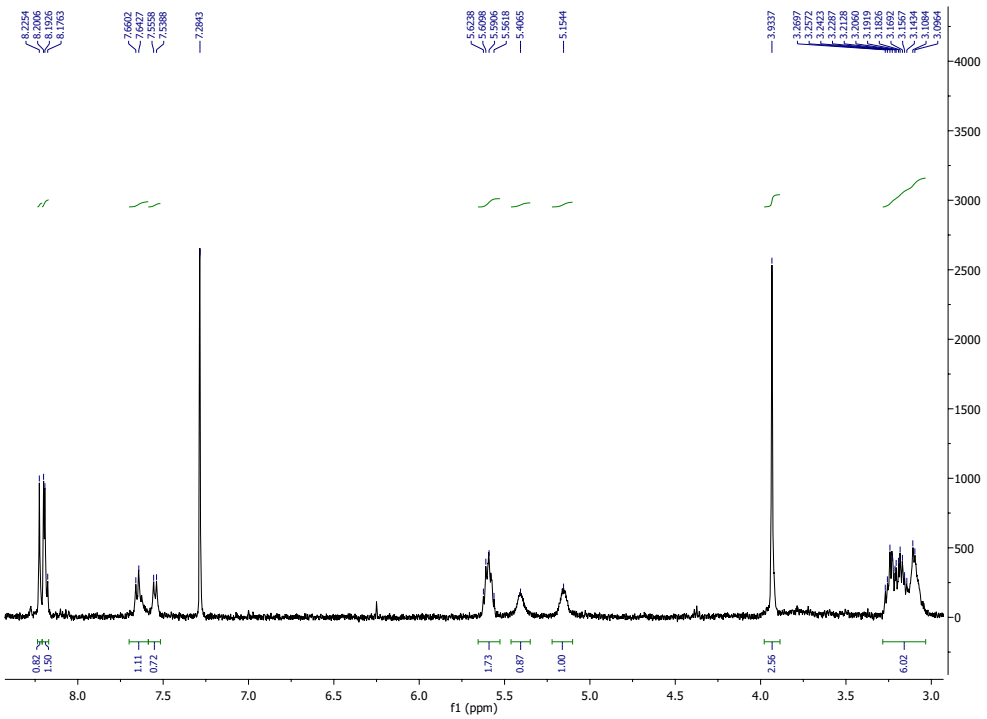


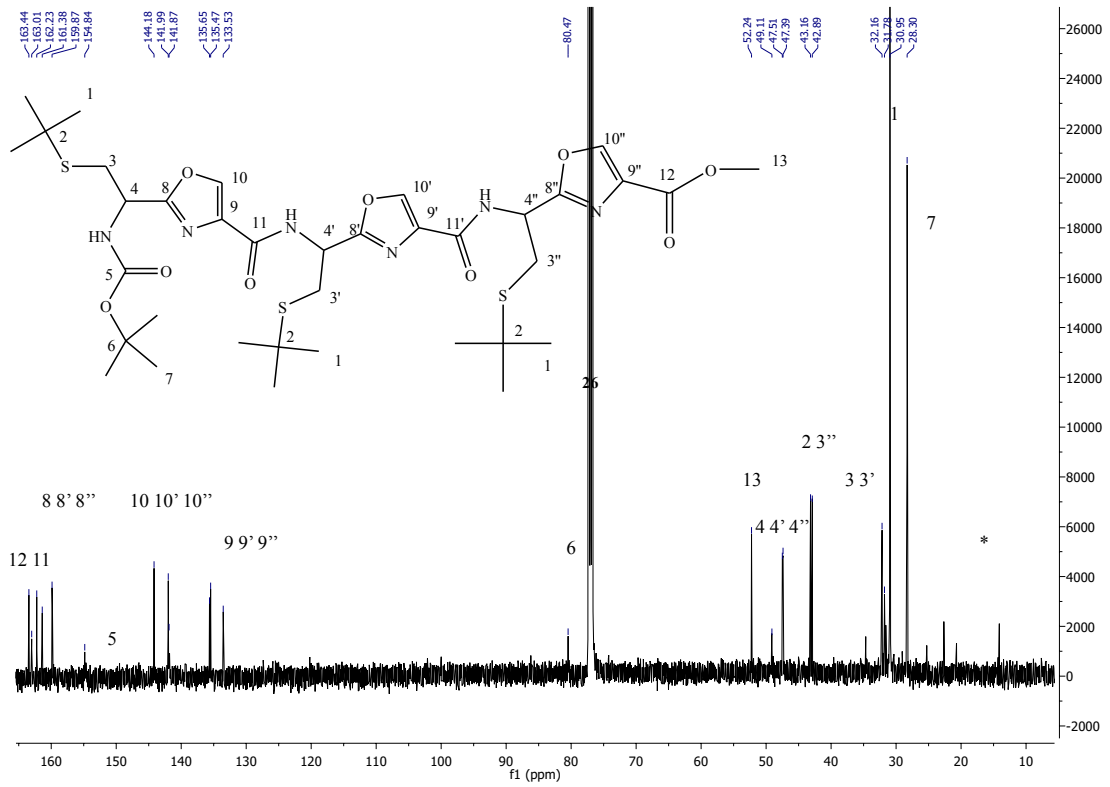


Appendix 57: ^{13}C NMR Boc-dioxazole-COOH (25).



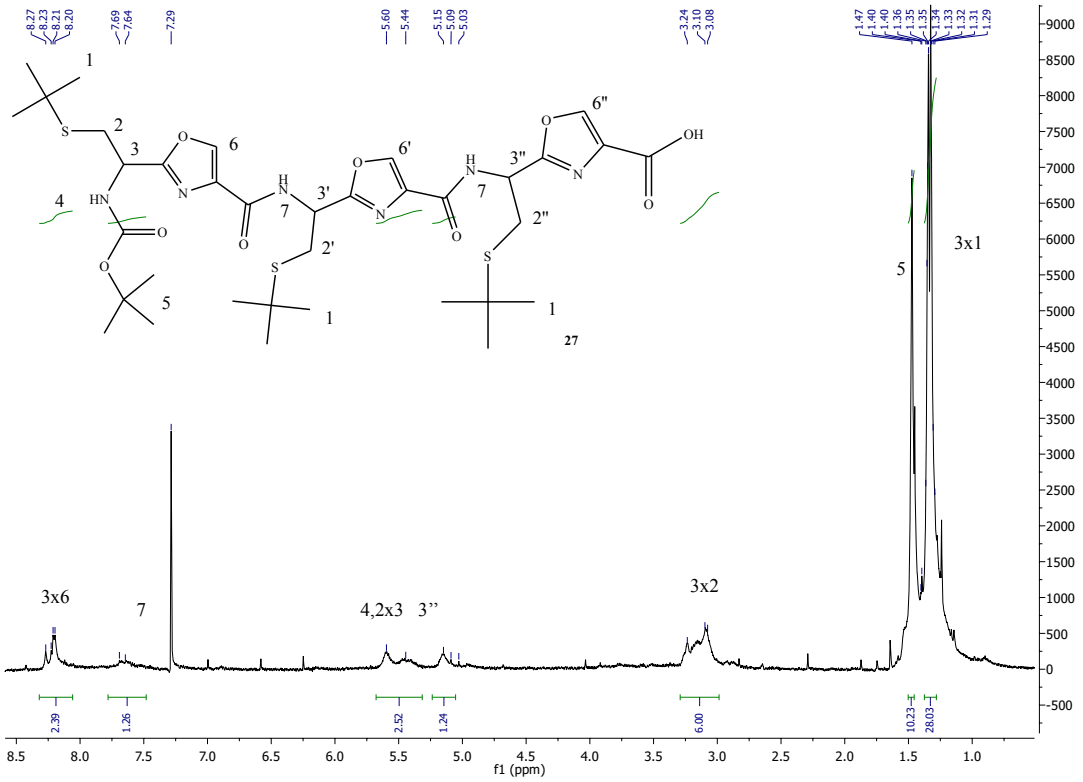
Appendix 58: ^{1}H NMR Boc-trioxazole-OMe (26).



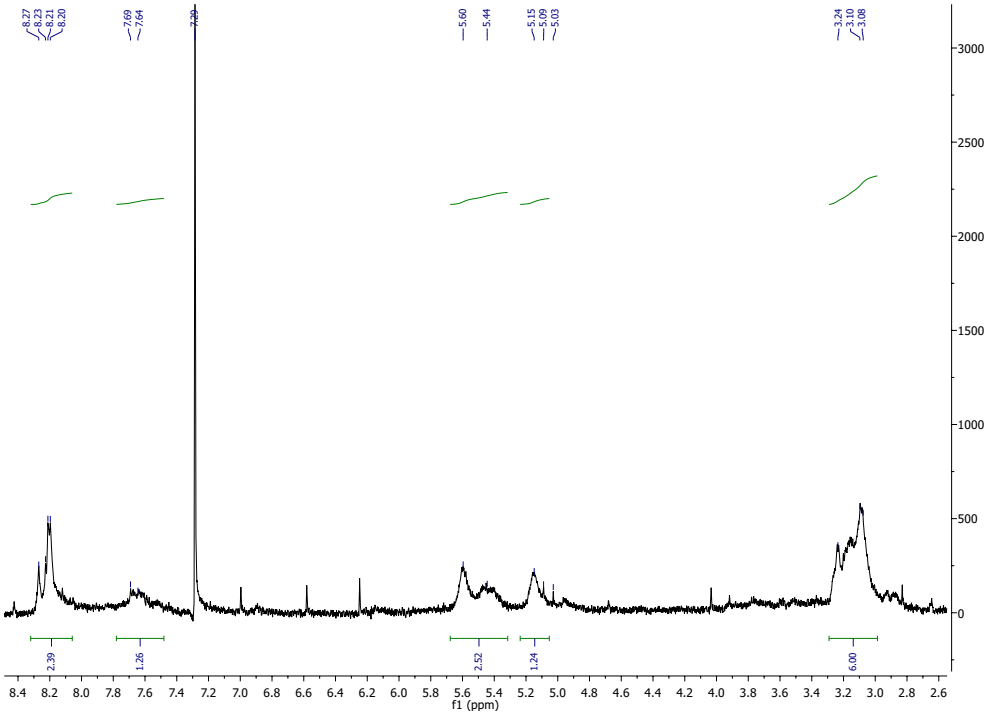


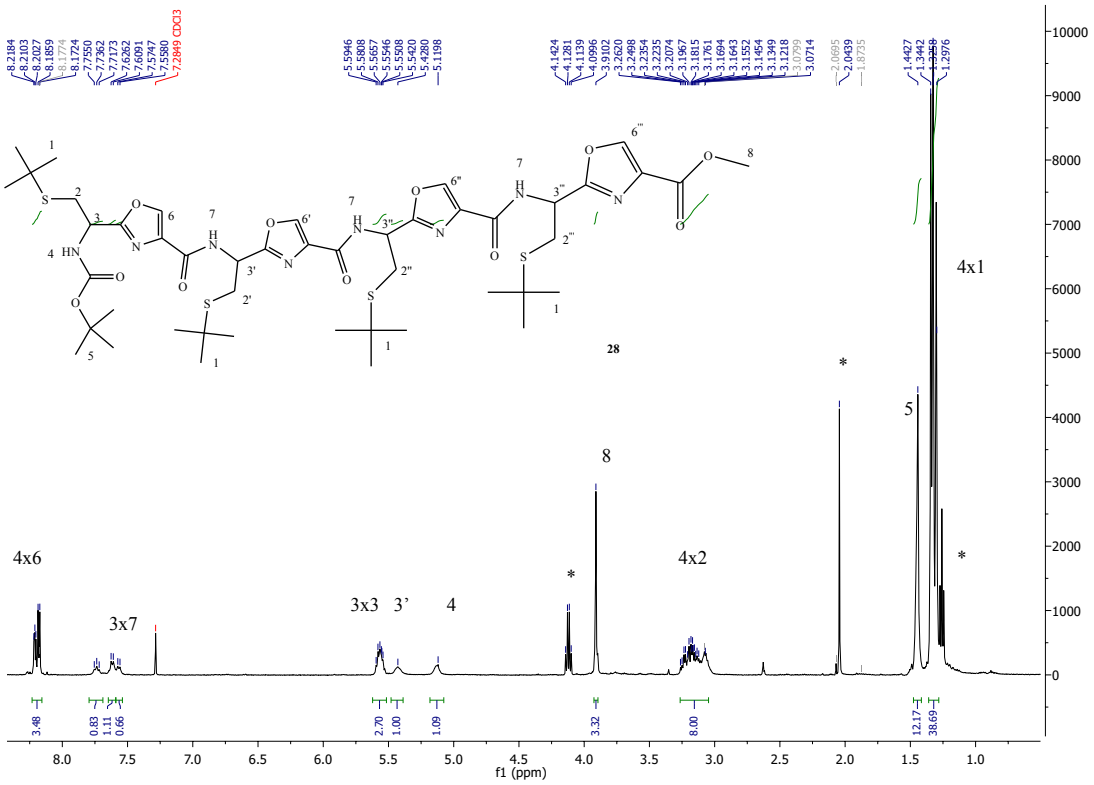
Appendix 59: ^{13}C NMR Boc-trioxazole-OMe (26).

- Peaks due to hexane

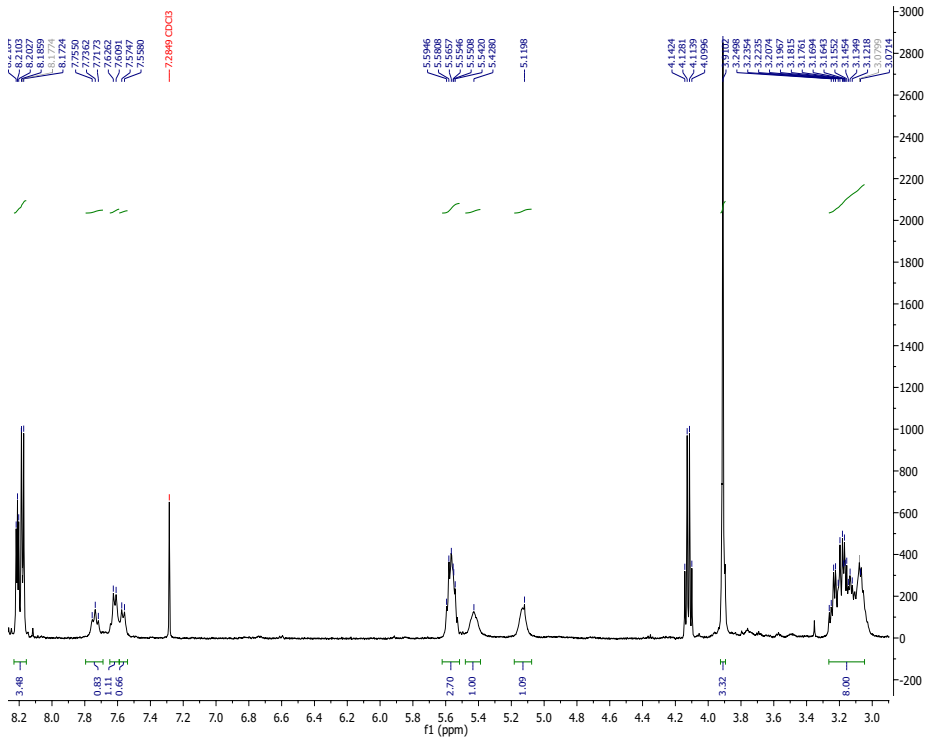


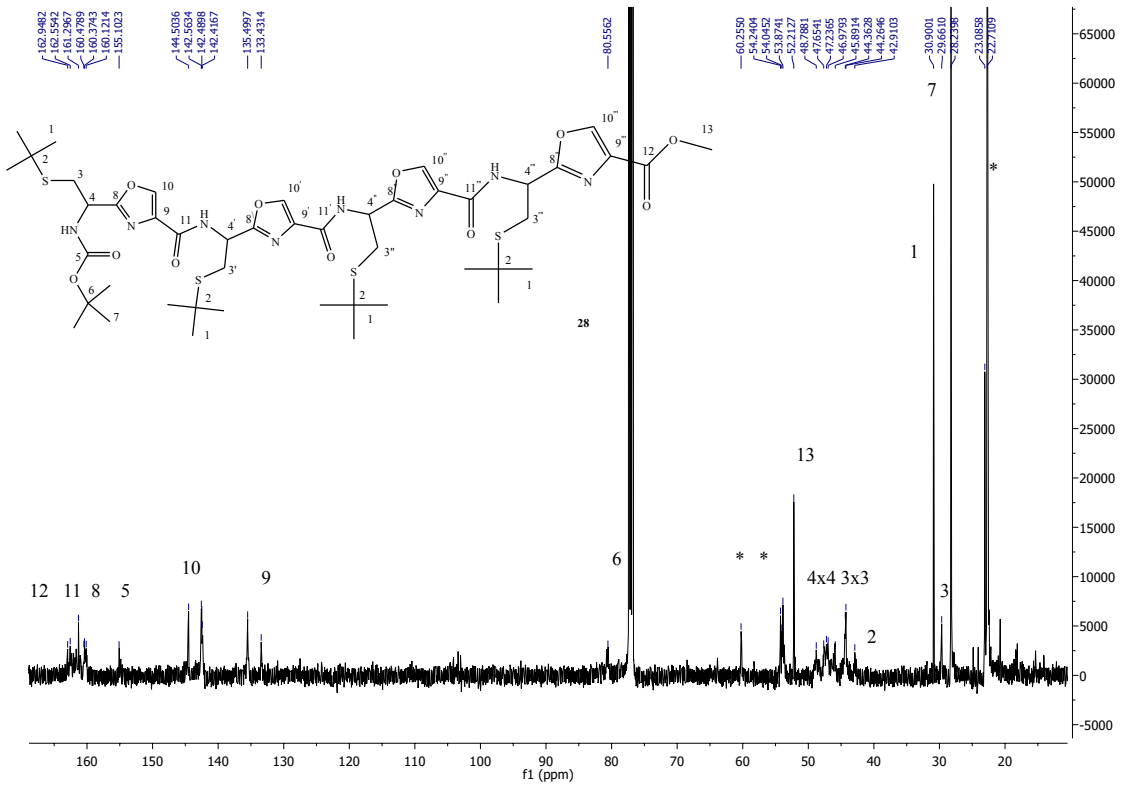
Appendix 60: ^{1}H NMR Boc-trioxazole-COOH (27).





Appendix 61: ^{1}H NMR Boc-tetraoxazole-OMe (28).

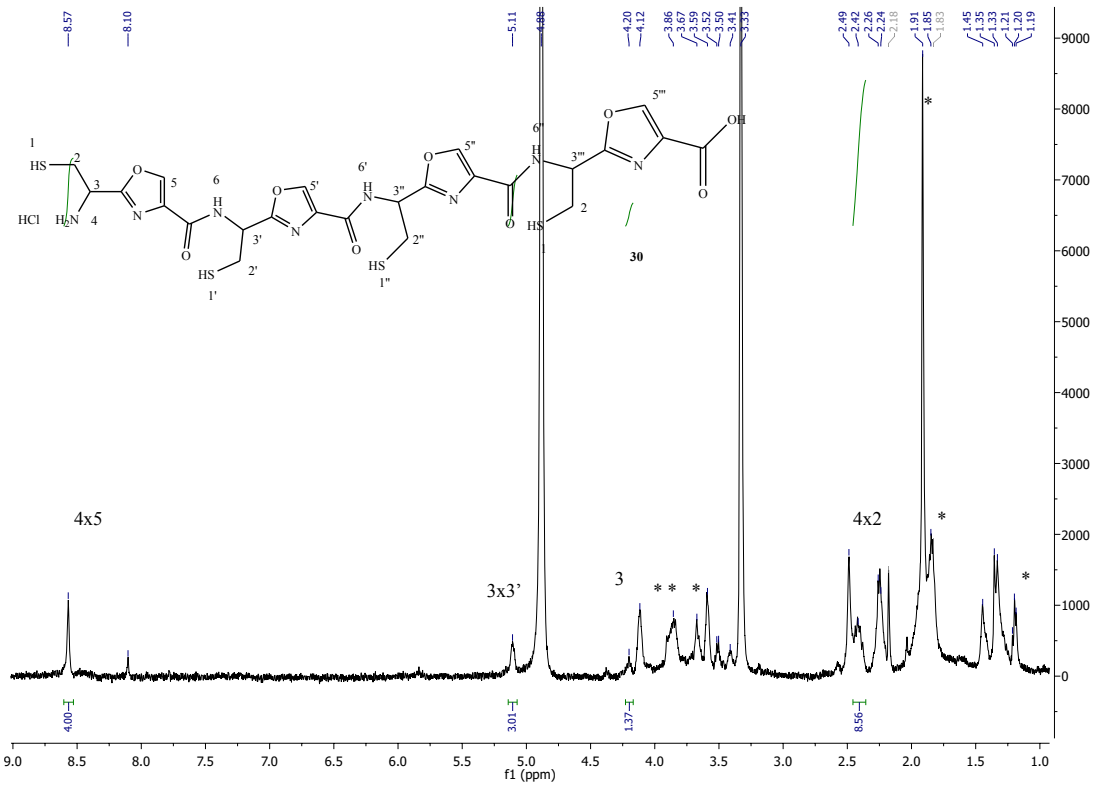




Appendix 62: ^{13}C NMR Boc-tetraoxazole-OMe (28).

* Peaks at 1.26, 2.05 and 4.12 ppm due to EtOAc in ^{1}H NMR

* Peak at 22.71ppm due to Hexane and at 60.25 ppm for EtOAc and at 53.89 ppm for DCM



Appendix 63: ^{1}H NMR HCl.H_2N-tetraoxazole-COOH (30).

* Peaks at 1.18, 3.51ppm for diethyl ethe, at 1.8, 3.8 ppm for tetrahydrofuran, and at 3.66 ppm due to dioxane

* The singlet peak at 1.9 ppm might be from $C(\underline{C}H_3)_3Cl$ that came from t-Bu cleavage